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(54) Title: PROSTATE CANCER-RELATED COMPOSITIONS, METHODS AND KITS BASED ON DNA MACROARRAY PROTEMICS PLATFORMS

(57) Abstract: The invention relates to novel nucleic acids encoding a mammalian PCAM-1 gene, and proteins encoded thereby, whose expression is increased in certain diseases, disorders, or conditions, including, but not limited to, prostate cancer. The invention further relates to methods of detecting and treating prostate cancer, comprising modulating or detecting PCAM-1 expression and/or production and activity of PCAM-1 poly peptide. Further, the invention relates to novel assays for the identification of DNA-binding proteins and the double-stranded oligonucleotide sequences that specifically bind with them.

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A

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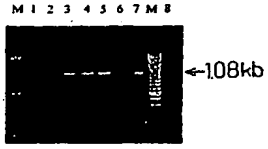

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PROSTATE CANCER-RELATED COMPOSITIONS, METHODS, AND KITS BASED ON DNA MACROARRAY PROTEOMICS PLATFORMS

BACKGROUND OF THE INVENTION

The prior art suggests that DNA binding proteins play an important role in certain diseases, disorders or conditions. More specifically, there are many reports demonstrating a connection between overexpression of genes encoding DNA binding ribosomal proteins and cancer (Chiao et al., 1992, Mol. Carcinog. 5:219-231; Fernandez-Pol et al., 1993, J. Biol. Chem. 268:21198-211204; Fernandez-Pol et al., 1994, Cell Growth & Differentiation 5:821-825; Fernandez-Pol, 1996, Anticancer Res. 16:2177-2186; Chan et al., 1996, Biochem. and Biophys. Res. Comm. 228:141-147; Chan et al., 1996, Biochem. and Biophys. Res. Comm. 225:952-956; Wool, 1996, Trends in Biochemical Sciences 21:164-165; Wool et al., 1995, Biochem. Cell Biol. 73:933-947; and Vaarala et al., 1998, Int. J. Cancer 78:27-32). For instance, Chiao et al. (1992, Mol. Carcinog. 5:219-231) determined that expression of the DNA binding S2-ribosomal protein was elevated in head and neck cancer, but S2 was barely detectable in normal tissue. Based upon these studies, it is widely believed that ribosomal proteins play a role in elevating protein synthesis and can thereby play a role in cancer.

Alternatively, it has been proposed that specific leucine zipper sequence motifs or other motifs characteristic of numerous ribosomal proteins may be mutated and that the mutant protein can then bind to nucleic acids whereas the wild type protein does not (Fernandez-Pol et al., 1996, Anticancer Res. 16:2177-2186; Wool, 1996, Trends in Biochemical Sciences 21:164-165; Wool, 1997, In: The ribosomal RNA and Group I introns, pp. 153-178, Green and Schroeder, eds., R.G. Landes Co., Austin, TX). Studies have proposed that DNA binding proteins either function as a nuclease, control ligation, or regulate gene transcription or translation in cancer cells. For example, the rat ribosomal protein S3a is identical to

the product of the rat v-fos transformation effector gene (Chan et al., 1996, Biochem. Biophys. Res. Comm. 228:141-147). S3a is involved in initiation of protein synthesis and is also related to proteins involved in the regulation of growth and the cell cycle (Chan et al., 1996, Biochem. and Biophys. Res. Comm. 228:141-147). Likewise, the rat ribosomal protein L10 is homologous to a DNA-binding protein to a putative Wilm's tumor suppressor gene (Chan et al., 1996, Biochem. Biophys. Res. Comm. 225:952-956). These studies suggest that mutant ribosomal-like proteins may be prognostic or diagnostic for cancer and play an important role in regulating the behavior of cancer cells.

DNA binding proteins are also believed to play a role in lymphoid tumors. The existence of chromosomal abnormalities in lymphoid tumors is well established. Chromosomal translocations associated with T cell acute lymphoblastic leukemia (T-ALL) have led to the identification of several potential oncogenes (Rabbitts, 1991, Cell 67:641-644). Many of the T-ALL associated chromosomal translocations have been localized to the T-cell receptor (TCR) genes.

Recombination of the immunoglobulin gene takes place at early phase of B-lymphocyte differentiation. The V-(D)-J recombination that joins two or three germline segments (*i.e.*, variable-V; diversity-D; and joining-J) segments into a variable-gene exon by site specific recombination contributes to amplification of the V-region diversity. Comparison of the nucleotide sequences of the flanking regions of the V, D, and J segments has demonstrated that two common blocks of nucleotide sequences are conserved (Early et al., 1980, Cell 19:981-992), including a heptamer CACTGTG and a T-rich nonamer GGTTTTTGT, which are separated by a spacer sequence of either 12 or 23 bases. The homology between the heptamer-spacer-heptamer-nonamer sequences of the T-cell receptor and immunoglobulin genes suggests that these elements, commonly referred to as Break Point Cluster Region or BPCRs, play an important role in V-(D)-J recombination.

The prior art suggests that DNA binding protein(s) that recognize the conserved recombination signal sequence (RS) may be involved in the recombinational machinery that cleaves DNA at the juncture between the signal and coding region sequences and ligates the cleaved ends. Earliest reports disclosed RS proteins as being located in lymphoid cells (Aguilera et al., 1987, Cell 51:909-917; Halligan and Desiderio, 1987, Proc. Natl. Acad. Sci. USA 84:7019-7023;

Hamaguchi et al., 1989, Nucleic Acid Res. 17:9015-9026; and Mak, 1994, Nucleic Acid Res. 22:383-390). Different RS proteins have been identified more recently. For example, a DNA binding protein for kappaB binding and recognition component of the V(D)J recombination signal sequence has been identified. Activation of this family of transcription factors is thought to provide a mechanism by which oncogenic tyrosine kinases regulate genes with kappaB-controlled gene regulatory elements.

Studies on T cell abnormalities have been particularly informative with respect to recombinase involvement, especially with respect to breakpoints within the chromosome band 11p13. It seems that recombinase is responsible for abnormal chromosomal unions, because often both reciprocal translocated chromosomes have N-region nucleotide addition which is a hallmark of recombinase activity (Alt and Baltimore, 1982, Proc. Natl. Acad. Sci. USA 79:4118-4223). These translocations are regarded as mutations of the normal chromosomal joining process.

In sum, the mechanism(s) by which chromosomal abnormalities associated with rearranging genes come about and the role of DNA binding enzymes involved in the normal antigen receptor gene rearrangement (*i.e.*, recombinases) (Croce, 1987, Cell 49:155-169), albeit well-studied, are still poorly understood. Thus, identification of new BPCRs and new recombinases is needed, especially for understanding non-lymphoid type diseases and solid cancer development.

Further, although prior studies suggest that DNA binding proteins are associated with and/or mediate certain diseases, disorders or conditions, very few of these proteins have been identified (*e.g.*, to date, none have been identified in solid cancers) and their role(s) in the disease process is poorly understood. This is so despite the fact that there are various prior art assays for identification of DNA binding proteins (*e.g.*, Weissman et al., 2000, U.S. Patent No. 6,066,452; Edwards et al., 2000, U.S. Patent No. 6,010,849; Edwards et al., 1999, U.S. Patent No. 5,869,241; Sukhatme, 1999, U.S. Patent No. 5,866,325). Thus, there is a long-felt need for a simple, effective assay for the identification of DNA binding proteins and their cognate duplex DNA binding partners.

In addition, despite the potential usefulness of DNA binding proteins in the diagnosis and the development of therapeutics, there are few, if any, diagnostics and therapeutics based on DNA binding proteins or their cognate binding DNA duplexes.

Moreover, although prostate cancer is one of the leading causes of cancer-related mortality and morbidity in men, there are few effective diagnostics and therapeutics for this disease, and none are based on detection of a DNA binding protein, including proteins which bind BPCR's. There have been approximately 450 partially characterized tissue markers identified in the scientific literature, but only one has developed as a clinical marker approved by the FDA (*i.e.*, prostate specific antigen, or PSA and it's derivatives. Development of markers for the early detection of cancers, such as, but not limited to, prostate cancer is essential to improved treatment of cancer.

With respect to prostate cancer, it is generally believed that serum prostate specific antigen (PSA) levels are neither sensitive nor specific for identification of patients with prostate cancer (Garnick and Fair, 1998, Scientific Amer. December:75-83). It has been estimated that as many as 25% of men with prostate cancer have normal PSA levels. Thus, development of more sensitive and specific assays for cancer, including prostate cancer, is imperative. Further, non-invasive and inexpensive urine-based screening assays, which would enable implementation through mass community screening programs or in routine clinical examinations, would be particularly useful in diagnosis and treatment of cancers, including prostate cancer.

Thus, there is a long felt and acute need for identification and characterization of DNA binding proteins and the cognate duplex DNA molecules they specifically bind with for the development of diagnostics and therapeutics for diseases, disorders or conditions associated with altered expression of a DNA binding protein. Further, there is a long-felt and acute need for improved diagnostics and therapeutics related to cancer, including prostate cancer. The present invention meets these needs.

BRIEF SUMMARY OF THE INVENTION

The invention includes an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof.

The invention also includes an isolated nucleic acid encoding a mammalian prostate cancer marker 1, and homologs, variants, mutants and fragments thereof.

The invention includes an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, wherein said nucleic acid shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1).

The invention further includes an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, wherein the nucleic acid further comprises a nucleic acid encoding a tag polypeptide covalently linked thereto.

The invention further includes an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, wherein the nucleic acid further comprises a nucleic acid encoding a tag polypeptide covalently linked thereto, wherein said tag polypeptide is selected from the group consisting of a myc tag polypeptide, a glutathione-S-transferase tag polypeptide, a green fluorescent protein tag polypeptide, a myc-pyruvate kinase tag polypeptide, a His6 tag polypeptide, an influenza virus hemagglutinin tag polypeptide, a flag tag polypeptide, and a maltose binding protein tag polypeptide.

The invention includes an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said nucleic acid further comprising a nucleic acid encoding a promoter/regulatory sequence operably linked thereto.

The invention also includes a vector comprising a isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof. In one aspect, said vector further comprises a nucleic acid specifying a promoter/regulatory sequence operably linked thereto. In another aspect, said isolated nucleic acid encoding a mammalian prostate cancer marker 1 is expressed when introduced into a cell.

The invention includes a recombinant cell comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof.

The invention includes a recombinant cell comprising a vector comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof.

The invention includes a recombinant cell comprising a vector comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention also includes an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation.

In one aspect, the isolated nucleic acid shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1).

In another aspect, the isolated nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

In yet another aspect, the isolated nucleic acid is expressed when introduced into a cell.

The invention includes a vector comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation, wherein said isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1).

The invention further includes a vector comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation, said isolated nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto, further wherein said isolated nucleic acid is expressed when introduced into a cell.

The invention includes a recombinant cell comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation.

The invention also includes a recombinant cell comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation, wherein said isolated nucleic acid shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1).

The invention includes a recombinant cell comprising a vector comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation, wherein said isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1).

The invention includes a recombinant cell comprising a vector comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation, said isolated nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto, further wherein said isolated nucleic acid is expressed when introduced into a cell.

The invention includes an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2.

In one aspect, the nucleic acid further comprises a nucleic acid encoding a tag polypeptide covalently linked thereto.

In another aspect, the tag polypeptide is selected from the group consisting of a myc tag polypeptide, a glutathione-S-transferase tag polypeptide, a green fluorescent protein tag polypeptide, a myc-pyruvate kinase tag polypeptide, a His6 tag polypeptide, an influenza virus hemagglutinin tag polypeptide, a flag tag polypeptide, and a maltose binding protein tag polypeptide.

The invention includes an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said nucleic acid further comprising a nucleic acid encoding a promoter/regulatory sequence operably linked thereto.

The invention includes a vector comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2.

In one aspect, the vector further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

In another aspect, the isolated nucleic acid encoding a mammalian prostate cancer marker 1 is expressed when introduced into a cell.

The invention includes a recombinant cell comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2.

The invention also includes a recombinant cell comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, the nucleic acid further comprising a nucleic acid encoding a tag polypeptide covalently linked thereto.

The invention includes a recombinant cell comprising a vector comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2.

The invention includes a recombinant cell comprising a vector comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, the nucleic acid further comprising a nucleic acid encoding a tag polypeptide covalently linked thereto.

In one aspect, the vector is expressed when introduced into said cell.

The invention includes an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said complementary nucleic acid being in an antisense orientation.

In one aspect, the complementary nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention includes a vector comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said complementary nucleic acid being in an antisense orientation, the complementary nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention includes a vector comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said complementary nucleic acid being in an antisense orientation, the complementary nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto, wherein said isolated nucleic acid is expressed when introduced into a cell.

The invention includes an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said complementary nucleic acid being in an

antisense orientation, wherein said nucleic acid shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1).

In one aspect, the isolated nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention further includes a vector comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said complementary nucleic acid being in an antisense orientation, and wherein said nucleic acid shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1).

The invention includes a vector comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said complementary nucleic acid being in an antisense orientation, wherein said nucleic acid shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1), and the isolated nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

In one aspect, the isolated nucleic acid is expressed when introduced into a cell.

The invention includes a recombinant cell comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said complementary nucleic acid being in an antisense orientation, wherein said nucleic acid shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1).

The invention includes a recombinant cell comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97%

sequence identity with the amino acid sequence SEQ ID NO:2, said complementary nucleic acid being in an antisense orientation, wherein said nucleic acid shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1), the isolated nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

In one aspect, the isolated nucleic acid is expressed in said cell.

The invention includes an isolated polypeptide comprising a mammalian prostate cancer marker 1.

In one aspect, the mammalian prostate cancer marker 1 shares at least about 97% sequence identity with an amino acid of SEQ ID NO:2.

The invention also includes an isolated nucleic acid that specifically binds with a prostate cancer marker 1 polypeptide.

In one aspect, the isolated nucleic acid is a double-stranded DNA.

In another aspect, the isolated nucleic acid comprises a nucleic acid sequence selected from the group consisting of a nucleic acid sequence CACGGATG (SEQ ID NO:5), a nucleic acid sequence CACAATGA (SEQ ID NO:6), a nucleic acid sequence CACAATG (SEQ ID NO:7), and a nucleic acid sequence CACAATGTTTTTGT (SEQ ID NO:8).

The invention includes an isolated nucleic acid that specifically binds with a mammalian leukemia cell break point cluster region binding protein.

In one aspect, the leukemia break point cluster region binding protein is selected from the group consisting of a Rag 1 protein and a Rag 2 protein.

In further aspect, the isolated nucleic acid comprises a double-stranded DNA, said DNA comprising a nucleic acid sequence selected from the group consisting of a nucleic acid sequence CACGGATG (SEQ ID NO:5), and a nucleic acid sequence CACAATGA (SEQ ID NO:6).

The invention includes an isolated nucleic acid that specifically binds with a prokaryotic break point cluster region binding protein.

In one aspect, the prokaryotic break point cluster region binding protein is selected from the group consisting of a RecA protein and a RecB protein.

The invention includes an isolated polypeptide comprising a mammalian prostate cancer marker 1, wherein the polypeptide specifically binds

with at least one of a nucleic acid selected from the group consisting of a nucleic acid consisting of the sequence CACGGATG (SEQ ID NO:5), a nucleic acid consisting of the sequence CACAATGA (SEQ ID NO:6), a nucleic acid consisting of the sequence CACAATG (SEQ ID NO:7), and a nucleic acid consisting of the sequence CACAATGTTTTTGT (SEQ ID NO:8).

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1.

In one aspect, the isolated enzymatic nucleic acid comprises a nucleic acid sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11).

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 comprises a nucleic acid having the sequence SEQ ID NO:1, or a portion thereof.

The invention includes a recombinant cell comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 comprises a nucleic acid having the sequence SEQ ID NO:1, or a portion thereof.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said enzymatic nucleic acid comprises binding arms and further wherein said binding arms comprise a sequence complementary to SEQ ID NO:1, or a portion thereof.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention includes a vector an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1.

The invention includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

The invention includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

The invention includes a recombinant cell comprising a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1.

The invention includes a recombinant cell comprising a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention includes a recombinant cell comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 comprises a nucleic acid having the sequence SEQ ID NO:1, or a portion thereof, wherein said enzymatic nucleic acid is expressed therein.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 comprises

a nucleic acid having the sequence SEQ ID NO:1, or a portion thereof, wherein said enzymatic nucleic acid is in a hairpin motif.

The invention further includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 comprises a nucleic acid having the sequence SEQ ID NO:1, or a portion thereof, wherein said enzymatic nucleic acid is in a hammerhead motif.

The invention further includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 comprises a nucleic acid having the sequence SEQ ID NO:1, or a portion thereof, wherein said enzymatic nucleic acid comprises a stem II region of length greater than or equal to 2 base pairs.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1).

The invention includes a recombinant cell comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1). In one aspect, the isolated nucleic acid is expressed therein.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1), said enzymatic nucleic acid comprising binding arms wherein said binding arms comprise a sequence complementary to SEQ ID NO:1, or a portion thereof.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer

marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1), said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1). In one aspect, the enzymatic nucleic acid is expressed when introduced into a cell.

The invention includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1), said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto. In one aspect, the enzymatic nucleic acid is expressed when introduced into a cell.

The invention includes a recombinant cell comprising a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1).

The invention also includes a recombinant cell comprising a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1), said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto. In one aspect, the enzymatic nucleic acid is expressed in the cell.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1), and wherein said enzymatic nucleic acid is in a hairpin motif.

The invention also includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1), and wherein said enzymatic nucleic acid is in a hammerhead motif.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1), wherein said enzymatic nucleic acid comprises a stem II region of length greater than or equal to 2 base pairs.

The invention further includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2.

The invention includes a recombinant cell comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2. In one aspect, the enzymatic nucleic acid is expressed in the cell.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded

by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, wherein said enzymatic nucleic acid is in a hairpin motif.

The invention also includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, wherein said enzymatic nucleic acid is in a hammerhead motif.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, wherein said enzymatic nucleic acid comprises a stem II region of length greater than or equal to 2 base pairs.

The invention further includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 consisting of a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11).

The invention includes a recombinant cell comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 consisting of a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11).

In one aspect, the isolated nucleic acid is expressed in the cell.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 consisting of a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11), said enzymatic nucleic acid comprising binding arms wherein said binding arms comprise a sequence complementary to SEQ ID NO:1, or a portion thereof.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 consisting of a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11), said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention also includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 consisting of a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11).

The invention includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 consisting of a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11), said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention also includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 consisting of a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11), said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

The invention includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 consisting of a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11), said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

The invention further includes a recombinant cell comprising a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 consisting of a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11).

The invention includes a recombinant cell comprising a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 consisting of a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence

AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11), said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said enzymatic nucleic acid comprising binding arms wherein said binding arms comprise a sequence complementary to SEQ ID NO:1, or a portion thereof.

The invention includes an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2.

The invention also includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid

encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

The invention includes a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

The invention includes a recombinant cell comprising a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2.

The invention includes a recombinant cell comprising a vector comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

The invention includes an antibody that specifically binds with a mammalian prostate cancer marker 1 polypeptide, or a fragment thereof.

The invention also includes an antibody that specifically binds with a mammalian prostate cancer marker 1 polypeptide, or a fragment thereof, wherein said antibody is selected from the group consisting of a polyclonal antibody, a

monoclonal antibody, a humanized antibody, a chimeric antibody, and a synthetic antibody.

The invention includes a composition comprising an antibody that specifically binds with a mammalian prostate cancer marker 1 polypeptide, or a fragment thereof, and a pharmaceutically-acceptable carrier.

The invention also includes a composition comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, and a pharmaceutically-acceptable carrier.

The invention includes a composition comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation, and a pharmaceutically-acceptable carrier.

The invention also includes a composition comprising an isolated polypeptide comprising a mammalian prostate cancer marker 1, and a pharmaceutically-acceptable carrier.

The invention further includes a composition comprising an isolated nucleic acid that specifically binds with a prostate cancer marker 1 polypeptide and a pharmaceutically-acceptable carrier.

The invention includes a composition comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, and a pharmaceutically-acceptable carrier.

The invention also includes a composition comprising an antibody that specifically binds with a mammalian prostate cancer marker 1 polypeptide, or a fragment thereof , and a pharmaceutically-acceptable carrier.

The invention includes a transgenic non-human mammal comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof.

The invention also includes a transgenic non-human mammal comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1.

The invention further includes a transgenic non-human mammal comprising an isolated nucleic acid complementary to an isolated nucleic acid

encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation.

The invention includes a method of treating a disease mediated by mal-expression of a prostate cancer marker 1 in a mammal. The method comprises administering to a human afflicted with a disease mediated by mal-expression of a prostate cancer marker 1 expression-inhibiting amount of at least one substance selected from the group consisting of an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, and an antibody that specifically binds with a mammalian prostate cancer marker 1.

In one aspect, the disease is prostate cancer.

In another aspect, the mammal is selected from the group consisting of a human and a dog.

In yet another aspect, the method further comprises administering an enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a polypeptide selected from a group consisting of a vascular epithelial growth factor 1 (VEGF-1) and a metalloproteinase 2 (MMP-2).

The invention includes a method of diagnosing prostate cancer in a mammal, said method comprising obtaining a biological sample from said mammal, assessing the level of PCAM-1 in said biological sample, and comparing the level of PCAM-1 in said biological sample with the level of PCAM-1 in a biological sample obtained from a like mammal not afflicted with prostate cancer, wherein a higher level of PCAM-1 in said biological sample from said mammal compared with the level of PCAM-1 in said biological sample from said like mammal is an indication that said mammal is afflicted with prostate cancer, thereby diagnosing prostate cancer in said mammal.

In one aspect, the mammal is selected from the group consisting of a human and a dog.

In another aspect, the biological sample is selected from the group consisting of a prostate tissue sample, a blood sample, a urine sample, a sputum sample, a peritoneal cavity fluid sample, a perineal cavity fluid sample, a pleural

cavity fluid sample, a semen sample, a prostatic fluid sample, a stool sample, and a bone marrow sample.

The invention includes a method of diagnosing prostate cancer in a mammal, said method comprising obtaining a biological sample from said mammal, assessing the level of antibody that specifically binds with prostate cancer marker 1 in said biological sample, and comparing the level of antibody that specifically binds with prostate cancer marker 1 in said biological sample with the level of antibody that specifically binds with prostate cancer marker 1 in a biological sample obtained from a like mammal not afflicted with prostate cancer, wherein a higher level of antibody that specifically binds with prostate cancer marker 1 in said biological sample from said mammal compared with the level of antibody that specifically binds with prostate cancer marker 1 in said biological sample from said like mammal is an indication that said mammal is afflicted with prostate cancer, thereby diagnosing prostate cancer in a mammal.

In one aspect, the mammal is selected from the group consisting of a human and a dog.

In another aspect, the biological sample is selected from the group consisting of a prostate tissue sample, a blood sample, a urine sample, a sputum sample, a peritoneal cavity fluid sample, a perineal cavity fluid sample, a pleural cavity fluid sample, a semen sample, a prostatic fluid sample, a stool sample, and a bone marrow sample.

The invention also includes a method of identifying a test compound that affects expression of prostate cancer marker 1 in a cell The method comprises contacting a cell with a test compound and comparing the level of prostate cancer marker 1 expression in said cell with the level of prostate cancer marker 1 expression in an otherwise identical cell not contacted with said test compound, wherein a higher or lower level of prostate cancer marker 1 expression in said cell contacted with said test compound compared with the level of prostate cancer marker 1 expression in said otherwise identical cell not contacted with said test compound is an indication that said test compound affects expression of prostate cancer marker 1 in a cell.

In one aspect, the invention includes a compound identified by the method.

The invention includes a method of identifying a compound that reduces expression of prostate cancer marker 1 in a cell. The method comprises contacting a cell with a test compound and comparing the level of prostate cancer marker 1 expression in said cell with the level of prostate cancer marker 1 expression in an otherwise identical cell not contacted with said test compound, wherein a lower level of prostate cancer marker 1 expression in said cell contacted with said test compound compared with the level of prostate cancer marker 1 expression in said otherwise identical cell not contacted with said test compound is an indication that said test compound reduces expression of prostate cancer marker 1 in a cell. The invention includes a compound identified by this method.

The invention includes a method of identifying a compound that increases expression of prostate cancer marker 1 in a cell. The method comprises contacting a cell with a test compound and comparing the level of prostate cancer marker 1 expression in said cell with the level of prostate cancer marker 1 expression in an otherwise identical cell not contacted with said test compound, wherein a higher level of prostate cancer marker 1 expression in said cell contacted with said test compound compared with the level of prostate cancer marker 1 expression in said otherwise identical cell not contacted with said test compound is an indication that said test compound increases expression of prostate cancer marker 1 in a cell. The invention includes a compound identified by this method.

The invention includes a method of identifying a compound that affects binding of a prostate cancer marker 1 with a double-stranded nucleic acid that specifically binds with prostate cancer marker 1. The method comprises comparing the level of prostate cancer marker 1 binding with a double-stranded nucleic acid that specifically binds with a prostate cancer marker 1 in the presence of a compound with the level of prostate cancer marker 1 binding with said double-stranded nucleic acid that specifically binds with a prostate cancer marker 1 in the absence of said compound, wherein a higher or lower level of prostate cancer marker 1 binding with said double-stranded nucleic acid that specifically binds with a prostate cancer marker 1 in the presence of said compound compared with the level of prostate cancer marker 1 binding with said double-stranded nucleic acid that specifically binds with a prostate cancer marker 1 in the absence of said compound is an indication that said compound affects binding of a prostate cancer

marker 1 with a double-stranded nucleic acid that specifically binds with prostate cancer marker 1, thereby identifying a compound that affects binding of a prostate cancer marker 1 with a double-stranded nucleic acid that specifically binds with prostate cancer marker 1.

The invention includes a compound identified by this method.

In one aspect, the double-stranded nucleic acid that specifically binds with prostate cancer marker 1 has a sequence selected from the group consisting of a sequence CACGGATG (SEQ ID NO:5), a sequence CACAATGA (SEQ ID NO:6), a sequence CACAATG (SEQ ID NO:7), and a sequence CACAATGTTTTTGT (SEQ ID NO:8).

In another aspect, the prostate cancer marker 1 has a sequence that shares greater than about 97% amino acid homology with a sequence SEQ ID NO:2.

The invention includes a method of monitoring the treatment of a human having prostate cancer. The method comprises:

(a) assessing the level of prostate cancer marker 1 in a first biological sample obtained from said human to determine an initial level of prostate cancer marker 1;

(b) administering an anti-prostate cancer therapy to said human;

(c) assessing the level of prostate cancer marker 1 in a second otherwise identical biological sample obtained from said human during or after said therapy;

(d) comparing said level of prostate cancer marker 1 in said first biological sample with said level of prostate cancer marker 1 in said second biological sample; and

(e) correlating any reduction in level of prostate cancer marker 1 with the effectiveness of said anti-prostate cancer therapy,

thereby monitoring the treatment of a human having prostate cancer.

In one aspect, the method further comprises repeating (b) through (e) during the course of said human's illness, anti-prostate cancer therapy, or any period or portion thereof.

In another aspect, the level of prostate cancer marker 1 is assessed using a method selected from the group consisting of a method of detecting a nucleic acid encoding a prostate cancer marker 1, and a method of detecting a prostate cancer marker 1.

The invention includes a method of monitoring the treatment of a human having prostate cancer. The method comprises:

(a) assessing the level of prostate cancer marker 1 in a first biological sample obtained from said human to determine an initial level of prostate cancer marker 1;

(b) administering an anti-prostate cancer therapy to said human;

(c) assessing the level of prostate cancer marker 1 in a second otherwise identical biological sample obtained from said human during or after said therapy;

(d) comparing said level of prostate cancer marker 1 in said first biological sample with said level of prostate cancer marker 1 in said second biological sample; and

(e) correlating any reduction in level of prostate cancer marker 1 with the effectiveness of said anti-prostate cancer therapy,

thereby monitoring the treatment of a human having prostate cancer, wherein said method of detecting a prostate cancer marker 1 is selected from the group consisting of a method of detecting an antibody that specifically binds with a prostate cancer marker 1, and a method of detecting binding of a double-stranded nucleic acid that specifically binds with a prostate cancer maker 1 wherein said nucleic acid is selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8.

The invention includes a kit for alleviating a disease mediated by mal-expression of prostate cancer marker 1 in a mammal. The kit comprises a prostate cancer marker 1 expression-inhibiting amount of at least one molecule selected from the group consisting of an antibody that specifically binds with prostate cancer marker 1, an isolated nucleic acid complementary to a nucleic acid

encoding a prostate cancer marker 1, said complementary nucleic acid being in an antisense orientation, and an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof.

In one aspect, the disease is prostate cancer.

In another aspect, the isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 comprises a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11).

In yet another aspect, the kit further comprises an enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a polypeptide selected from a group consisting of a vascular epithelial growth factor 1 (VEGF-1) and a metalloproteinase 2 (MMP-2).

The invention includes a kit for treating a disease mediated by mal-expression of prostate cancer marker 1 in a mammal. The kit comprises a prostate cancer marker 1 expression-inhibiting amount of at least one molecule selected from the group consisting of an antibody that specifically binds with prostate cancer marker 1, an isolated nucleic acid complementary to a nucleic acid encoding a prostate cancer marker 1, said complementary nucleic acid being in an antisense orientation, and an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof.

The invention includes a kit for assessing the level of prostate cancer marker 1 in a sample. The kit comprises a molecule that specifically binds with prostate cancer marker 1 polypeptide or with nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof.

The invention includes a kit for assessing the level of prostate cancer marker 1 in a sample. The kit comprises a molecule that specifically binds with

prostate cancer marker 1 polypeptide or with nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof, wherein said molecule that specifically binds with a prostate cancer marker 1 polypeptide is selected from the group consisting of an antibody that specifically binds with prostate cancer marker 1, and a double-stranded nucleic acid that specifically binds with prostate cancer marker 1.

The invention also includes a kit for assessing the level of prostate cancer marker 1 in a sample. The kit comprises a molecule that specifically binds with prostate cancer marker 1 polypeptide or with nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof, wherein said nucleic acid encoding prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid having the sequence SEQ ID NO:1.

The invention includes a kit for assessing the level of prostate cancer marker 1 in a sample. The kit comprises a molecule that specifically binds with prostate cancer marker 1 polypeptide or with nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof, wherein said prostate cancer marker 1 polypeptide shares greater than about 97% sequence identity with an amino acid sequence SEQ ID NO:2.

The invention includes a kit for assessing the level of prostate cancer marker 1 in a sample. The kit comprises a molecule that specifically binds with prostate cancer marker 1 polypeptide or with nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof, wherein said molecule that specifically binds with a prostate cancer marker 1 polypeptide is selected from the group consisting of an antibody that specifically binds with prostate cancer marker 1, and a double-stranded nucleic acid that specifically binds with prostate cancer marker 1, wherein said double-stranded nucleic acid that specifically binds with prostate cancer marker 1 comprises a sequence selected from the group consisting of a sequence CACGGATG (SEQ ID NO:5), a sequence CACAATGA (SEQ ID NO:6), a sequence CACAATG (SEQ ID NO:7), and a sequence CACAATGTTTTTGT (SEQ ID NO:8).

The invention also includes a kit for detecting prostate cancer marker 1 in a mammal. The kit comprises a molecule that specifically binds with prostate cancer marker 1 polypeptide or with a nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof.

In one aspect, the mammal is selected from the group consisting of a dog and a human.

In another aspect, the molecule that specifically binds with a prostate cancer marker 1 polypeptide is selected from the group consisting of an antibody that specifically binds with a prostate cancer marker 1, and a double-stranded nucleic acid that specifically binds with prostate cancer marker 1.

In yet another aspect, the double-stranded nucleic acid that specifically binds with prostate cancer marker 1 comprises a sequence selected from the group consisting of a sequence CACGGATG (SEQ ID NO:5), a sequence CACAATGA (SEQ ID NO:6), a sequence CACAATG (SEQ ID NO:7), and a sequence CACAATGTTTTTGT (SEQ ID NO:8).

The invention also includes a kit for detecting prostate cancer marker 1 in a mammal. The kit comprises a molecule that specifically binds with prostate cancer marker 1 polypeptide or with a nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof, wherein said molecule that specifically binds with a nucleic acid encoding a prostate cancer marker 1 is selected from the group consisting of a nucleic acid complementary with a nucleic acid sharing greater than 98% sequence identity with sequence SEQ ID NO:1.

The invention includes a Monte Carlo-like screening assay for identification of a double-stranded oligonucleotide that specifically binds with a DNA-binding protein. The assay comprises:

(a) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide comprises a random core nucleotide sequence flanked by a known sequence comprising at least two basepairs; and

(b) detecting any oligonucleotide member of said set that specifically binds with a DNA-binding protein, thereby identifying a double-stranded oligonucleotide that specifically binds with a DNA-binding protein.

The invention includes an isolated double-stranded oligonucleotide that specifically binds with a DNA-binding protein identified by this assay.

In one aspect, the detecting of (b) comprises a method selected from the group consisting of an electrophoretic mobility shift assay and a method of detecting a double-stranded oligonucleotide bound with a polypeptide.

In another aspect, the random core nucleotide sequence comprises from about 3 to 12 basepairs.

In yet another aspect, the double-stranded oligonucleotide ranges in length from about 7 to 16 basepairs.

In yet a further aspect, the random core nucleotide sequence comprises a length selected from the group consisting of 7 basepairs, 8 basepairs, and 9 basepairs.

The invention includes a Monte Carlo-like screening assay for identification of a double-stranded oligonucleotide that specifically binds with a DNA-binding protein. The assay comprises:

(a) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide comprises a random core nucleotide sequence flanked by a known sequence comprising at least two basepairs; and

(b) detecting any oligonucleotide member of said set that specifically binds with a DNA-binding protein, thereby identifying a double-stranded oligonucleotide that specifically binds with a DNA-binding protein, the assay further comprising

(c) identifying the sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein;

(d) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide consists of the known flanking sequence identified in (c), said oligonucleotide further comprising an additional known such that the unknown random core sequence consists of one less unknown basepair than the sequence identified in (c), and repeating (b) and (c).

In one aspect, the assay further comprises repeating steps (c), (b) and (c) until the entire sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein is identified.

The invention includes a method of identifying a double stranded-oligonucleotide that specifically binds with a DNA-binding protein associated with a tumor. The method comprises

(a) producing a semi-random double-stranded oligonucleotide set wherein each double-stranded oligonucleotide comprises a random core nucleotide sequence flanked by a known sequence comprising at least two basepairs;

(b) mixing a double-stranded oligonucleotide member of said set with a sample containing a mixture comprising DNA-binding proteins prepared from a tumor cell or tissue under conditions in which one or more of said double-stranded oligonucleotides in said set specifically binds a DNA-binding protein;

(c) mixing an identical double-stranded oligonucleotide member of said set with an otherwise identical sample containing a mixture comprising DNA-binding proteins prepared from an otherwise identical cell or tissue not comprising a tumor under conditions in which one or more of said double-stranded oligonucleotides in said set specifically binds with a DNA-binding protein;

(d) detecting any specific oligonucleotide-protein binding in (a) and (b); and

(e) identifying any double-stranded oligonucleotide that specifically binds with a DNA-binding protein in (b) but which does not specifically bind with a DNA-binding protein in (c), thereby identifying a double-stranded oligonucleotide that specifically binds with a DNA-binding protein associated with a tumor.

The invention includes an isolated double-stranded oligonucleotide identified by this method.

The invention includes a method of identifying a double stranded-oligonucleotide that specifically binds with a DNA-binding protein associated with a tumor. The method comprises

(a) producing a semi-random double-stranded oligonucleotide set wherein each double-stranded oligonucleotide comprises a random core nucleotide sequence flanked by a known sequence comprising at least two basepairs;

(b) mixing a double-stranded oligonucleotide member of said set with a sample containing a mixture comprising DNA-binding proteins prepared

from a tumor cell or tissue under conditions in which one or more of said double-stranded oligonucleotides in said set specifically binds a DNA-binding protein;

(c) mixing an identical double-stranded oligonucleotide member of said set with an otherwise identical sample containing a mixture comprising DNA-binding proteins prepared from an otherwise identical cell or tissue not comprising a tumor under conditions in which one or more of said double-stranded oligonucleotides in said set specifically binds with a DNA-binding protein;

(d) detecting any specific oligonucleotide-protein binding in (a) and (b); and

(e) identifying any double-stranded oligonucleotide that specifically binds with a DNA-binding protein in (b) but which does not specifically bind with a DNA-binding protein in (c), thereby identifying a double-stranded oligonucleotide that specifically binds with a DNA-binding protein associated with a tumor, further wherein said detecting of (d) comprises a method selected from the group consisting of an electrophoretic mobility shift assay and a method of detecting a labeled double-stranded oligonucleotide bound with a polypeptide.

The invention includes a method of identifying a double stranded-oligonucleotide that specifically binds with a DNA-binding protein associated with a tumor. The method comprises

(a) producing a semi-random double-stranded oligonucleotide set wherein each double-stranded oligonucleotide comprises a random core nucleotide sequence flanked by a known sequence comprising at least two basepairs;

(b) mixing a double-stranded oligonucleotide member of said set with a sample containing a mixture comprising DNA-binding proteins prepared from a tumor cell or tissue under conditions in which one or more of said double-stranded oligonucleotides in said set specifically binds a DNA-binding protein;

(c) mixing an identical double-stranded oligonucleotide member of said set with an otherwise identical sample containing a mixture comprising DNA-binding proteins prepared from an otherwise identical cell or tissue not comprising a tumor under conditions in which one or more of said double-stranded oligonucleotides in said set specifically binds with a DNA-binding protein;

(d) detecting any specific oligonucleotide-protein binding in (a) and (b); and

(e) identifying any double-stranded oligonucleotide that specifically binds with a DNA-binding protein in (b) but which does not specifically bind with a DNA-binding protein in (c), thereby identifying a double-stranded oligonucleotide that specifically binds with a DNA-binding protein associated with a tumor, further wherein said random core nucleotide sequence comprises from about 3 to 12 basepairs.

In one aspect, the double-stranded oligonucleotide ranges in length from about 7 to 16 basepairs.

In another aspect, the random core nucleotide sequence comprises a length selected from the group consisting of 7 basepairs, 8 basepairs, and 9 basepairs.

In yet another aspect, the method further comprises

(f) identifying the sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein in (e);

(g) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide consists of the known flanking sequence identified in (f), said oligonucleotide further comprising an additional known basepair adjacent to said unknown random core sequence such that said unknown random core sequence consists of one less unknown basepair than the sequence identified in (f); and

(h) repeating (b) and (e).

In another aspect, the method further comprises repeating (b) through (h) until the entire sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein is identified.

The invention includes a Monte Carlo-like screening assay for identification of a double-stranded DNA-binding protein. The assay comprises

(a) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide comprises a random core nucleotide sequence flanked by a known sequence comprising at least two basepairs; and

(b) detecting any DNA-binding protein that specifically binds with an oligonucleotide member of said set, thereby identifying a double-stranded DNA-binding protein.

The invention includes an isolated double-stranded DNA-binding protein identified by this assay.

In one aspect, the detecting of (b) comprises a method selected from the group consisting of an electrophoretic mobility shift assay and a method of detecting a double-stranded oligonucleotide bound with a polypeptide.

In another aspect, the random core nucleotide sequence comprises from about 3 to 12 basepairs.

In yet another aspect, the double-stranded oligonucleotide ranges in length from about 7 to 16 basepairs.

In a further aspect, the random core nucleotide sequence comprises a length selected from the group consisting of 7 basepairs, 8 basepairs, and 9 basepairs.

The invention includes a Monte Carlo-like screening assay for identification of a double-stranded DNA-binding protein. The assay comprises

(a) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide comprises a random core nucleotide sequence flanked by a known sequence comprising at least two basepairs; and

(b) detecting any DNA-binding protein that specifically binds with an oligonucleotide member of said set, thereby identifying a double-stranded DNA-binding protein, where the assay further comprises

(c) identifying the sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein;

(d) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide consists of said known flanking sequence identified in (c), said oligonucleotide further comprising an additional known such that the unknown random core sequence consists of one less unknown basepair than the sequence identified in (c), and repeating (b) and (c).

In one aspect, the assay further comprises repeating steps (c), (b) and (c) until the entire sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein is identified.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiment(s) which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown. In the drawings:

Figure 1A depicts the nucleic acid sequence (SEQ ID NO:1) of prostate cancer antigen marker 1 (PCAM-1).

Figure 1B depicts the amino acid sequence (SEQ ID NO:2) of PCAM-1.

Figure 2 is an image depicting an example of data obtained from a Monte Carlo-like screening assay comparing binding of the (γ-ATP)^{32}P-labeled CACGGATG probe (SEQ ID NO:5) (1 ng at 100,000 cpm) binding activity. Phosphoimaging was performed using ^{32}P-labeled (50,000 cpm/well) double stranded DNA oligonucleotide (8 basepairs) (CACGGATG [SEQ ID NO:5]) binding to crude protein (2μg protein/well) extracts on a nylon membrane filter. Crude protein extracts were prepared from human prostates, including: Lane (1) seminal vesicle; (2) BPH; (3) HGPIN; and cancer of (4) Gleason score 8; (5) Gleason score 7; and (4) Gleason score 5. Rows (a-c) and (d-f) show 3 replicates from 2 different cancers, respectively, for each stage or grade of cancer examined. Lane 7 contains crude protein extracts from (a-c) a lambda phage clone expressing recombinant PCAM-1 protein; and (d-f) PC-3 ML cells.

Figure 3 is a diagrammatic representation of the hammerhead PCAM-1 DNA ZYM 1 domain known in the art. Stem II can be 2 base-pair long. Conserved nucleotides are formatted in bold; N represents any nucleotide; H represents the nucleotides C, U and A; line segments represent either Watson Crick or reversed-Hoogsteen base pairs.

Figure 4 is a diagrammatic representation of the general structure of a hairpin DNA enzyme.

Figure 5A is an image depicting an agarose gel (1%) demonstrating total RNA (28S and 18S) isolated from normal (N) and prostate cancer (T) tissue from the same patients (*i.e.*, matching samples). The data disclosed demonstrate

that whole RNA (*i.e.*, 28S and 18S RNA) was not digested by the PCAM-1 DNA ZYM-1 (ZYM, SEQ ID NO:9, 31 bp) or a random nucleotide sequence (R, 31 bp). The image depicts whole RNA (5 μg/well) treated with: (lanes 1, 2) vehicle, and the DNA ZYMs at dosages of 1 μg /ml (lanes 3, 4); 2 μg /ml(lanes 5, 6); and 4 μg /ml (lanes 7,8) for 4 hours.

Figure 5B is an image depicting RT-PCR and PCR products (*i.e.*, approximately 895 bp) generated (35 cycles) using a PCAM-1 mRNA primer specific for the 5' translational start site of the PCAM-1 gene, *e.g.*, 5'-TACCCCTTGGCGCCACCGAAGGCGCCT-3' (SEQ ID NO:12) and 5'-TCCGCGGAAGCCACCGCGGTTCCCCAT-3' (SEQ ID NO:13), from whole RNA isolated from normal, BPH and cancerous human prostate tissue (5 μg RNA/sample). The data disclosed demonstrate that the primer fails to amplify the PCAM-1 mRNA (895 bp) isolated from (lanes 1, 3, 11) normal human prostate tissue or (lane 7) human BPH tissue; but did amplify a 895 bp transcript present in whole RNA isolated from human prostate tumor tissue (lanes 4, 5, 6, 8, 9, 10, 12, 14, 16). Whole RNA samples (5 μg total) were pre-incubated with the PCAM-1 DNA ZYM-1 for 2 hours (SEQ ID SEQ NO:9) at 1 μg /ml (lane 13), and 2 μg /ml (lane 15) or with a random DNA sequence (31 basepair) at 2 μg /ml (lane 14)and 5 μg /ml (lane 16).

Figure 6 is an image depicting a Northern blot of RNA isolated from (lane 1) PC-3 ML cells; and (lanes 2-5) dissected human prostate cancer glands from 4 different patients. Total RNA was isolated using TRIzol Reagent and Poly (A)+ RNA isolated from the total RNA. RNA was electrophoresed on a denaturing agarose gel. The Northern blot was carried out with the ^{32}P-labeled cDNA specific to PCAM-1 according to Sambrook et al. (1989, *supra*). Each lane was were loaded with 2 μg RNA.

Figure 7 is an image of a gel depicting RT-PCR of mRNA isolated from (lanes 1-3) three human prostate tumors; (lane 4) LNCaP cells; (lane 5) PC-3 ML cells; (lane 6) NPTX-1532 cells (normal cells); (lane 7) λPhage clone expressing recombinant PCAM-1. M- marker lanes. The 1.08 Kb sequence of PCAM-1 mRNA was amplified using forward and reverse primer sequences, including: 5'-TACCCCTTGGCGCCACCGAAGGCGCCTCCAAAGCCG-3' (SEQ ID NO:3) and 5'-

CGGCTTTGGAGGCGCCTTCGGTGGCGCCAAGGGGTA-3' (SEQ ID NO:4), respectively. Thermal cycling was at 94°C for 1 minute, followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1.5 minutes. "M" indicates the 100 bp DNA ladder. Lane 8 is a negative control where SUPERSCRIPT II reverse transcriptase was omitted from the reverse transcription reaction.

Figure 8 is an image depicting RT-PCR of mRNA isolated from human prostate glands microdissected from (lanes 1-3) three Gleason score 8 foci; (lanes 4-6) three Gleason score 6 foci; (lanes 7-9) three HGPIN foci; and (lanes 10-12) three BPH foci. M- marker lanes. The 1.08 Kb sequence of PCAM-1 mRNA was amplified using forward and reverse primer sequences, including: 5'-TACCCCTTGGCGCCACCGAAGGCGCCTCCAAAGCCG-3' (SEQ ID NO:3); and 5'-CGGCTTTGGAGGCGCCTTCGGTGGCGCCAAGGGGTA-3'(SEQ ID NO:4), respectively. Thermal cycling was at 94°C for 1 minute, followed by 60 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1.5 minutes.

Figure 9 is a graph depicting cell survival curves after 1-5 days of PC-3 ML cells (series 3) non-transfected; or overnight with different CMV constructs, including series: (1) CMV-vector alone (10 μg/ml); and (4-7) CMV-PCAM-1 ribozyme at 3, 5, 8 and 10 μg/ml, respectively. (Series 2) NPTX-1532 cells transfected with the CMV-PCAM-1 ribozyme construct (10 μg/ml).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a novel "Monte Carlo-like" assay for identification of DNA binding proteins and their cognate DNA molecule binding partner. Further, the invention relates to the identification of a novel DNA binding protein, and the cognate DNA molecule that specifically binds therewith. That is, the invention provides the nucleic and amino acid sequences of the novel DNA binding protein, designated PCAM-1 (prostate cancer marker 1, previously designated PSTF-1). The invention further relates to a nucleic acid enzyme complementary to PCAM-1 (termed PCAM-1 ZYM-1), which cleaves PCAM-1, and methods of treating cancer using the same.

The present invention relates to PCAM-1-based assays that easily and efficiently assess the presence or absence of prostate cancer in a patient by assessing the level of PCAM-1 in a biological sample compared to the level of

PCAM-1 in an otherwise identical biological sample obtained from a human known not to have prostate cancer. The disclosure of International Application No. PCT/US00/25981 is hereby incorporated herein by reference in its entirety.

It has also been discovered, as disclosed herein, that expression of PCAM-1 is increased in prostate cancer tissue and in urine of prostate cancer patients. Further, expression of PCAM-1 is particularly increased in nuclear protein extracts from prostate cancer tumors compared with the level of PCAM-1 in matching seminal vesicle (SV), benign prostatic hyperplasia (BPH) or high-grade prostatic intraepithelial neoplasm (HGPIN) foci. Moreover, the data disclosed herein demonstrate a correlation between the level of PCAM-1 protein in a biological sample and the Gleason Score (GS) of the prostate cancer examined thereby indicating that PCAM-1 can be a stage specific prostate cancer marker useful for proper staging of prostate cancer.

Further, the present invention relates to modulation of PCAM-1 expression and methods of treating cancer, including prostate cancer, mediated thereby. The data disclosed herein suggests that expression of PCAM-1 is associated with prostate cancer and the invention provides methods of diagnosis as well as for the development of therapeutics useful for treating and diagnosing diseases, disorders or conditions associated with altered expression of PCAM-1, including prostate cancer.

Definitions

As used herein, each of the following terms has the meaning associated with it in this section.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, the term "adjacent" is used to refer to nucleotide sequences which are directly attached to one another, having no intervening nucleotides. By way of example, the pentanucleotide 5'-AAAAA-3' is adjacent the trinucleotide 5'-TTT-3' when the two are connected thus: 5'-AAAAATTT-3' or 5'-TTTAAAAA-3', but not when the two are connected thus: 5'-AAAAACTTT-3'.

As used herein, amino acids are represented by the full name thereof, by the three letter code corresponding thereto, or by the one-letter code corresponding thereto, as indicated in the following table:

Full Name	Three-Letter Code	One-Letter Code
Aspartic Acid	Asp	D
Glutamic Acid	Glu	E
Lysine	Lys	K
Arginine	Arg	R
Histidine	His	H
Tyrosine	Tyr	Y
Cysteine	Cys	C
Asparagine	Asn	N
Glutamine	Gln	Q
Serine	Ser	S
Threonine	Thr	T
Glycine	Gly	G
Alanine	Ala	A
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Methionine	Met	M
Proline	Pro	P
Phenylalanine	Phe	F
Tryptophan	Trp	W

As used herein, to "alleviate" cancer means reducing the severity of one or more symptoms of prostate cancer. This can include, but is not limited to, reducing the level of PCAM-1 expressed in a cell or tissue, reducing the level of cell proliferation, reducing or increasing the level of PCAM-1 in the bloodstream or in the urine or other bodily fluid, and the like, in a patient, compared with the level of PCAM-1 in the patient prior to or in the absence of the method of treatment.

By the term "altered expression of PCAM-1," as used herein, is meant that the level of expression of a PCAM-1 in a cell is detectably higher or

lower than the level of expression of PCAM-1 in an otherwise identical cell where the otherwise identical cell is obtained from normal tissue that does not exhibit any detectable disease, disorder or condition associated with or mediated by expression of PCAM-1, such as, but not limited to, prostate cancer, other cancers and degenerative disorders such as osteoporosis, immune suppressive disorders or inflammatory disorders, and the like.

"Antisense" refers particularly to the nucleic acid sequence of the non-coding strand of a double stranded DNA molecule encoding a protein, or to a sequence which is substantially homologous to the non-coding strand. As defined herein, an antisense sequence is complementary to the sequence of a double stranded DNA molecule encoding a protein. It is not necessary that the antisense sequence be complementary solely to the coding portion of the coding strand of the DNA molecule. The antisense sequence may be complementary to regulatory sequences specified on the coding strand of a DNA molecule encoding a protein, which regulatory sequences control expression of the coding sequences.

By the term "applicator" as the term is used herein, is meant any device including, but not limited to, a hypodermic syringe, a pipette, and the like, for administering the PCAM-1 nucleic acid, protein, and/or composition of the invention to a mammal.

"Biological sample," as that term is used herein, means a sample obtained from an animal that can be used to assess the level of expression of a PCAM-1, the level of PCAM-1 protein present, or both. Such a sample includes, but is not limited to, a blood sample, a prostate biopsy, a urine sample, prostatic fluid, semen, lymph fluid, perineal cavity fluid sample, a peritoneal cavity fluid sample, pleural cavity fluid sample, a bone marrow sample, a salivary gland fluid, and a seminal vesicle tissue sample.

"Break point cluster region," as used herein, refers to nucleic acid sequences associated with a chromosomal translocation site, such as, but not limited, those identified in studies relating to leukemia.

By "candidate anti-PCAM-1 drug," as the term is used herein, is meant a compound that when contacted with a cell, reduces the level of expression of a nucleic acid encoding a PCAM-1 in the cell compared with the level of PCAM-1 expression in that cell prior to contacting the cell with the compound or which

reduces the level of expression in the cell compared with the level of PCAM-1 expression in an otherwise identical cell which is not contacted with the compound.

By "complementary to a portion or all of the nucleic acid encoding PCAM-1" is meant a sequence of nucleic acid which does not encode a PCAM-1 protein. Rather, the sequence which is being expressed in the cells is identical to the non-coding strand of the nucleic acid encoding a PCAM-1 protein and thus, does not encode PCAM-1 protein.

The terms "complementary" and "antisense" as used herein, are not entirely synonymous. "Antisense" refers particularly to the nucleic acid sequence of the non-coding strand of a double stranded DNA molecule encoding a protein, or to a sequence which is substantially homologous to the non-coding strand. "Complementary" as used herein refers to the broad concept of subunit sequence complementarity between two nucleic acids, *e.g.*, two DNA molecules. When a nucleotide position in both of the molecules is occupied by nucleotides normally capable of base pairing with each other, then the nucleic acids are considered to be complementary to each other at this position. Thus, two nucleic acids are complementary to each other when a substantial number (at least 50%) of corresponding positions in each of the molecules are occupied by nucleotides which normally base pair with each other (*e.g.*, A:T and G:C nucleotide pairs). As defined herein, an antisense sequence is complementary to the sequence of a double stranded DNA molecule encoding a protein. It is not necessary that the antisense sequence be complementary solely to the coding portion of the coding strand of the DNA molecule. The antisense sequence may be complementary to regulatory sequences specified on the non-coding (*i.e.*, intron) or coding (*i.e.*, exon) strand of a DNA molecule encoding a protein, which regulatory sequences control expression of the coding sequences.

A "coding region" of a gene consists of the nucleotide residues of the coding strand of the gene (*i.e.*, exon) and the nucleotides of the non-coding strand of the gene which are homologous with or complementary to, respectively, the coding region of an mRNA molecule which is produced by transcription of the gene.

A "coding region" of an mRNA molecule also consists of the nucleotide residues of the mRNA molecule which are matched with an anticodon

region of a transfer RNA molecule during translation of the mRNA molecule or which encode a stop codon. The coding region may thus include nucleotide residues corresponding to amino acid residues which are not present in the mature protein encoded by the mRNA molecule (*e.g.*, amino acid residues in a protein export signal sequence).

By the term "consensus", as used herein, is meant a nucleic acid sequence which has been re-sequenced to resolve un-called bases, or which has been extended using RT-PCR extension kit (such as, *e.g.*, that available from Perkin Elmer, Norwalk, CT) in the 5' and/or 3' direction and re-sequenced, or which has been assembled from the overlapping sequences of more that one derived clone (or which have been both extended and assembled).

A "non-coding" region of a gene consists of the nucleotide residues of the gene (*i.e.*, introns) including "leader sequences" which are important for mRNA binding to ribosomal proteins involved in mRNA translation to proteins.

By "consists essentially of" is meant that the active PCAM-1 DNA ZYMs contains an enzymatic center or core equivalent to those in the examples, as well as binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

"DNA-protein hybridization assays," as used herein, refers to a binding assays for identification of protein(s) which bind with specific DNA sequences and for assessing the amounts of protein binding to the DNA.

By "substrate binding arm" is meant that portion of a DNA enzyme which is complementary to (*i.e.*, able to base-pair with) a portion of its substrate. Generally, such complementary sequence is 100%, but can be less if desired. For example, as few as 10 bases out of 14 may be base-paired.

"Electrophoretic mobility shift assay" or "EMSA", as these terms are used herein, refers to a gel based assay for identification of protein(s) which bind specific DNA sequences and for assessing the amounts of protein binding to the DNA.

"Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and

mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

By "enhanced enzymatic activity" is meant to include activity measured in cells and/or *in vivo* where the activity is a reflection of both catalytic activity and PCAM-1 DNA ZYMs stability. In this invention, the product of these properties in increased or not significantly (less than 10 fold) decreased in vivo compared to an all PCAM-1 DNA ZYMs.

As used herein, "enzyme linked immuno-sandwich assay" is an antibody based assay for identification of protein and for measurements of protein levels in cell or tissue preparations.

By "enzymatic portion" is meant that part of the DNA enzyme essential for cleavage of an RNA substrate.

By "equivalent" RNA to PCAM-1 is meant to include those naturally occurring RNA molecules associated with cancer in various animals, including human. By "complementary" is meant a nucleic acid that can form hydrogen bond(s) with another RNA sequence by either traditional Watson-Crick or other non-traditional types of base-paired interactions.

"Expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (*e.g.*, naked or contained in

liposomes) and viruses (*e.g.*, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

A first region of an oligonucleotide "flanks" a second region of the oligonucleotide if the two regions are adjacent one another or if the two regions are separated by no more than about 1000 nucleotide residues, and preferably no more than about 100 nucleotide residues, and even more preferably, by no more than about 30 nucleotide residues.

As used herein, the term "fragment" as applied to a nucleic acid, may ordinarily be at least about 20 nucleotides in length, typically, at least about 50 nucleotides, more typically, from about 50 to about 100 nucleotides, preferably, at least about 100 to about 200 nucleotides, even more preferably, at least about 200 nucleotides to about 300 nucleotides, yet even more preferably, at least about 300 to about 350, even more preferably, at least about 350 nucleotides to about 500 nucleotides, yet even more preferably, at least about 500 to about 600, even more preferably, at least about 600 nucleotides to about 620 nucleotides, yet even more preferably, at least about 620 to about 650, and most preferably, the nucleic acid fragment will be greater than about 650 nucleotides in length.

As applied to a protein, a "fragment" of PCAM-1 is about 20 amino acids in length. More preferably, the fragment of a PCAM-1 is about 30 amino acids, even more preferably, at least about 40, yet more preferably, at least about 60, even more preferably, at least about 80, yet more preferably, at least about 100, even more preferably, about 100, and more preferably, at least about 110 amino acids in length.

A "genomic DNA" is a DNA strand which has a nucleotide sequence homologous with a gene. By way of example, both a fragment of a chromosome and a cDNA derived by reverse transcription of a mammalian mRNA are genomic DNAs.

A double-stranded oligonucleotide binds with "greatest affinity," as the term is used herein, when the double-stranded oligonucleotide produces the highest detectable signal indicating protein/DNA binding compared with any signal produced by any other member of the semi-random double-stranded oligonucleotide set of which the double-stranded oligonucleotide is a member.

"Homologous" as used herein, refers to the subunit sequence similarity between two polymeric molecules, *e.g.*, between two nucleic acid molecules, *e.g.*, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then they are homologous at that position. The homology between two sequences is a direct function of the number of matching or homologous positions, *e.g.*, if half (*e.g.*, five positions in a polymer ten subunits in length) of the positions in two compound sequences are homologous then the two sequences are 50% homologous, if 90% of the positions, *e.g.*, 9 of 10, are matched or homologous, the two sequences share 90% homology. By way of example, the DNA sequences 3'ATTGCC5' and 3'TATGGC share 50% homology.

As used herein, "homology" is used synonymously with "identity."

In addition, when the terms "homology" or "identity" are used herein to refer to the nucleic acids and proteins, it should be construed to be applied to homology or identity at both the nucleic acid and the amino acid sequence levels.

A first oligonucleotide anneals with a second oligonucleotide with "high stringency" or "under high stringency conditions" if the two oligonucleotides anneal under conditions whereby only oligonucleotides which are at least about 60%, more preferably at least about 65%, even more preferably at least about 70%, yet more preferably at least about 80%, and preferably at least about 90% or, more preferably, at least about 95% complementary anneal with one another. The stringency of conditions used to anneal two oligonucleotides is a function of, among other factors, temperature, ionic strength of the annealing medium, the incubation period, the length of the oligonucleotides, the G-C content of the oligonucleotides, and the expected degree of non-homology between the two oligonucleotides, if known. Methods of adjusting the stringency of annealing conditions are known (*see, e.g.*, Sambrook et al., 1989, In: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York).

The determination of percent identity between two nucleotide or amino acid sequences can be accomplished using a mathematical algorithm. For example, a mathematical algorithm useful for comparing two sequences is the algorithm of Karlin and Altschul (1990, Proc. Natl. Acad. Sci. USA 87:2264-2268),

modified as in Karlin and Altschul (1993, Proc. Natl. Acad. Sci. USA 90:5873-5877). This algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990, J. Mol. Biol. 215:403-410), and can be accessed, for example, at the National Center for Biotechnology Information (NCBI) world wide web site having the universal resource locator "http://www.ncbi.nlm.nih.gov/BLAST/". BLAST nucleotide searches can be performed with the NBLAST program (designated "blastn" at the NCBI web site), using the following parameters: gap penalty = 5; gap extension penalty = 2; mismatch penalty = 3; match reward = 1; expectation value 10.0; and word size = 11 to obtain nucleotide sequences homologous to a nucleic acid described herein. BLAST protein searches can be performed with the XBLAST program (designated "blastn" at the NCBI web site) or the NCBI "blastp" program, using the following parameters: expectation value 10.0, BLOSUM62 scoring matrix to obtain amino acid sequences homologous to a protein molecule described herein.

To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997, Nucleic Acids Res. 25:3389-3402). Alternatively, PSI-Blast or PHI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*id.*) and relationships between molecules which share a common pattern. When utilizing BLAST, Gapped BLAST, PSI-Blast, and PHI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. *See* http://www.ncbi.nlm.nih.gov.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

By "inhibit PCAM-1" is meant that the activity of PCAM-1 or level of RNAs encoded by PCAM-1 is reduced below that observed in the absence of the nucleic acid, particularly, inhibition with DNA enzymes preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave the RNA.

As used herein, the term "Gleason Score" refers to the pathological scoring system developed by Gleason et al. (1993, J. Urol. 149:1568-1576).

As used herein, the terms "gene" and "recombinant gene" refer to

nucleic acid molecules comprising an open reading frame encoding a polypeptide of the invention. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of a given gene. Alternative alleles can be identified by sequencing the gene of interest in a number of different individuals. This can be readily carried out by using hybridization probes to identify the same genetic locus in a variety of individuals. Any and all such nucleotide variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation and that do not alter the functional activity are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding proteins of the invention from other species (homologs), which have a nucleotide sequence which differs from that of the human proteins described herein are within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologs of a cDNA of the invention can be isolated based on their identity to human nucleic acid molecules using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. For example, a homolog of a human PCAM-1 protein of the invention can be isolated based on its hybridization with a nucleic acid molecule encoding all or part of human PCAM-1 under high stringency conditions.

As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the nucleic acid, peptide, and/or composition of the invention in the kit for effecting alleviation of the various diseases or disorders recited herein. Optionally, or alternately, the instructional material may describe one or more methods of alleviation the diseases or disorders in a cell or a tissue of a mammal. The instructional material of the kit of the invention may, for example, be affixed to a container which contains the nucleic acid, peptide, DNA enzyme and/or composition of the invention or be shipped together with a container which contains the nucleic acid, peptide, and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

An "isolated nucleic acid" refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, *e.g.*, a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, *e.g.*, the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, *e.g.*, RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. "A" refers to adenosine, "C" refers to cytidine, "G" refers to guanosine, "T" refers to thymidine, and "U" refers to uridine.

By the term "mal-expression of a PCAM-1 molecule," as used herein, is meant that the level of expression of a PCAM-1 in a cell is detectably higher or lower than the level of expression of PCAM-1 in an otherwise identical cell where the otherwise identical cell is obtained from normal tissue that does not exhibit any detectable disease, disorder or condition associated with or mediated by expression of PCAM-1, such as, but not limited to, prostate cancer, other cancers and degenerative disorders such as osteoporosis, immune suppressive disorders or inflammatory disorders, and the like, such that mal-expression is associated with or mediates a disease, disorder or condition.

"Monte-Carlo-like" screening assay, as used herein, refers to the production of random 7 basepair (bp), 8 bp, and 9 bp DNA sequences and protein binding assays employed to identify the 7 bp, 8 bp, and/or 9 bp sequence which binds a DNA-binding protein(s) produced by tumor tissue where the DNA-binding protein is either not produced or produced at a lower level in otherwise identical non-tumor tissue.

By describing two polynucleotides as "operably linked" is meant that a single-stranded or double-stranded nucleic acid moiety comprises the two polynucleotides arranged within the nucleic acid moiety in such a manner that at least one of the two polynucleotides is able to exert a physiological effect by which it is characterized upon the other. By way of example, a promoter operably linked to the coding region of a gene is able to promote transcription of the coding region.

Preferably, when the nucleic acid encoding the desired protein further comprises a promoter/regulatory sequence, the promoter/regulatory is positioned at the 5' end of the desired protein coding sequence such that it drives expression of the desired protein in a cell. Together, the nucleic acid encoding the desired protein and its promoter/regulatory sequence comprise a "transgene."

By "patient" is meant an organism which is a donor or recipient of explanted cells or the cells themselves. "Patient" also refers to an organism to which enzymatic nucleic acid molecules can be administered. Preferably, a patient is a mammal or mammalian cells. More preferably, a patient is a human or human cells or human tumors.

"PCAM-1", as used herein, refers to the amino acid sequences of purified recombinant or native "PCAM-1" protein obtained from any species or tissue or cells or from recombinant, synthetic or semi-synthetic sources. Preferably, the PCAM-1 is encoded by a nucleic acid that hybridizes with a nucleic acid having the sequence SEQ ID NO:1 under stringent conditions. Further, the PCAM-1 shares at least about 97% sequence identity with the amino acid sequence SEQ ID NO:2. Further, the PCAM-1 is over-expressed, *i.e.*, expressed at a level higher than the level present in a cell or tissue known not to have a disease, disorder, or condition. Also, the PCAM-1 is a cytoplasmic and nuclear protein of about 32 kDa with at least 5 specific mutations which distinguish PCAM-1 from the S2 gene. More preferably, the mRNA encoding PCAM-1 is cleaved by a PCAM-1 ZYM-1 ribonuclease disclosed herein, *e.g.*, a ribozyme having the sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11).

Unless otherwise indicated, "PCAM-1" and "prostate cancer marker 1" are used alternatively and refer to the polypeptide encoded by the nucleic acid encoding PCAM-1. Preferably, the nucleic acid encoding PCAM-1 shares greater than about 98% identity with the sequence SEQ ID NO:1, the PCAM-1 shares greater than about 97% with a protein having the amino acid sequence SEQ ID NO:2, or both. Further, the polypeptide preferably binds at least one double-stranded nucleic acid oligomer that specifically binds with PCAM-1, *e.g.*, the oligonucleotides having the sequence SEQ ID NOs:3-6.

As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

A "constitutive" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living human cell under most or all physiological conditions of the cell.

An "inducible" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living human cell substantially only when an inducer which corresponds to the promoter is present in the cell.

A "tissue-specific" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living human cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

A "polyadenylation sequence" is a polynucleotide sequence which directs the addition of a poly A tail onto a transcribed messenger RNA sequence.

A "polynucleotide" means a single strand or parallel and anti-parallel strands of a nucleic acid. Thus, a polynucleotide may be either a single-stranded or a double-stranded nucleic acid.

The term "nucleic acid" typically refers to large polynucleotides.

The term "oligonucleotide" typically refers to short polynucleotides, generally, no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (*i.e.*, A, T, G, C), this also includes an RNA sequence (*i.e.*, A, U, G, C) in which "U" replaces "T."

Conventional notation is used herein to describe polynucleotide sequences: the left-hand end of a single-stranded polynucleotide sequence is the 5'-end; the left-hand direction of a double-stranded polynucleotide sequence is referred to as the 5'-direction.

The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction. The DNA strand having the same sequence as an mRNA is referred to as the "coding strand"; sequences on the DNA strand which are located 5' to a reference point on the DNA are referred to as "upstream sequences"; sequences on the DNA strand which are 3' to a reference point on the DNA are referred to as "downstream sequences."

A "portion" of a polynucleotide means at least at least about twenty sequential nucleotide residues of the polynucleotide. It is understood that a portion of a polynucleotide may include every nucleotide residue of the polynucleotide.

"Primer" refers to a polynucleotide that is capable of specifically hybridizing to a designated polynucleotide template and providing a point of initiation for synthesis of a complementary polynucleotide. Such synthesis occurs when the polynucleotide primer is placed under conditions in which synthesis is induced, *i.e.*, in the presence of nucleotides, a complementary polynucleotide template, and an agent for polymerization such as DNA polymerase. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers are useful for many applications. A primer is complementary to the template to which it is designed to hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the template depends on the stringency of the hybridization conditions. Primers can be labeled with, *e.g.*, chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

"Probe" refers to a polynucleotide that is capable of specifically hybridizing to a designated sequence of another polynucleotide. A probe

specifically hybridizes to a target complementary polynucleotide, but need not reflect the exact complementary sequence of the template. In such a case, specific hybridization of the probe to the target depends on the stringency of the hybridization conditions. Probes can be labeled with, *e.g.*, chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

"Recombinant polynucleotide" refers to a polynucleotide having sequences that are not naturally joined together. An amplified or assembled recombinant polynucleotide may be included in a suitable vector, and the vector can be used to transform a suitable host cell.

A recombinant polynucleotide may serve a non-coding function (*e.g.*, promoter, origin of replication, ribosome-binding site, etc.) as well.

A "recombinant polypeptide" is one which is produced upon expression of a recombinant polynucleotide.

"Polypeptide" refers to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof linked via peptide bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Synthetic polypeptides can be synthesized, for example, using an automated polypeptide synthesizer.

The term "protein" typically refers to large polypeptides.

The term "peptide" typically refers to short polypeptides.

Conventional notation is used herein to portray polypeptide sequences: the left-hand end of a polypeptide sequence is the amino-terminus; the right-hand end of a polypeptide sequence is the carboxyl-terminus.

As used herein, the term "reporter gene" means a gene, the expression of which can be detected using a known method. By way of example, the *Escherichia coli lacZ* gene may be used as a reporter gene in a medium because expression of the lacZ gene can be detected using known methods by adding the chromogenic substrate *o*-nitrophenyl-β-galactoside to the medium (Gerhardt et al., eds., 1994, Methods for General and Molecular Bacteriology, American Society for Microbiology, Washington, DC, p. 574).

"PCAM-1-inhibiting amount," as used herein, means any amount of a substance or molecule that detectably decreases the level of PCAM-1 expression,

amount, and/or activity compared with the level of PCAM-1 expression, amount, and/or activity in the absence of the substance or molecule. Thus, any amount that mediates a detectable decrease in: the amount of PCAM-1 present and/or the level of PCAM-1 mRNA expression, is encompassed in the present invention. The assays by which these conditions are examined are well-known in the art and several are exemplified herein.

The term "PCAM-1 activity," as used herein, refers to the ability of a molecule or compound to ensure cell survival and growth, and the like.

By the term "PCAM-1 ZYM-1 activity," as used herein, refers to the ability of a molecule or compound to decrease expression of PCAM-1 compared with the level of PCAM-1 expression in the absence of PCAM-1 ZYM-1, the ability to hinder growth and/or decrease survival of malignant prostate cancer cells, *e.g.*, PC-3 ML cells, compared with the cell growth and survival of non-cancerous cells, *e.g.*, NPTX-1532 cells, BPH cells, human fibroblasts, and the like.

By "PCAM-1 DNA ZYM-1" it is meant a nucleic acid molecule which has complementary sequence in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave RNA in that target. That is, the enzymatic nucleic acid molecule is able to inter-molecularly cleave RNA and thereby inactivate a target RNA molecule. This complementary matching of sequence functions to allow sufficient hybridization of the enzymatic nucleic acid molecule to the target RNA to allow the cleavage to occur. One hundred percent complementary sequence is preferred, but complementary sequences as low as 50-75% may also be useful in this invention although, more preferably, the sequence is about 80% complementary.

The term "DNA enzymes" or "enzymatic nucleic acid" or "PCAM-1 DNA ZYM-1" specifically refers to a DNA sequence complementary to the PCAM-1 mRNA sequence. However, because they share a common functional capability, the term DNA ZYM is used interchangeably with phrases such as ribozymes, catalytic RNA, enzymatic RNA, catalytic DNA, nucleozyme, DNAzyme, RNA enzyme, endo-ribonuclease, mini-zyme, or leadzyme, oligozyme, as used in the art. All of these terminologies describe nucleic acid molecules with enzymatic activity.

By "associated with" and "mediated", used in the context of

diseases, disorders or conditions associated with and/or mediated by PCAM-1 mal-expression, is meant that the inhibition of PCAM-1 RNAs and thus reduction in the level respective protein activity, will relieve, to some extent, the symptoms of the disease, disorder or condition.

A "restriction site" is a portion of a double-stranded nucleic acid which is recognized by a restriction endonuclease.

A portion of a double-stranded nucleic acid is "recognized" by a restriction endonuclease if the endonuclease is capable of cleaving both strands of the nucleic acid at the portion when the nucleic acid and the endonuclease are contacted.

By the term "specifically binds," as used herein, is meant a compound, *e.g.*, a protein, a nucleic acid, an antibody, a ribozyme, and the like, which recognizes and binds a specific molecule, but does not substantially recognize or bind other molecules in a sample.

A first oligonucleotide anneals with a second oligonucleotide "with high stringency" if the two oligonucleotides anneal under conditions whereby only oligonucleotides which are at least about 75%, and preferably at least about 90% or at least about 95%, complementary anneal with one another. The stringency of conditions used to anneal two oligonucleotides is a function of, among other factors, temperature, ionic strength of the annealing medium, the incubation period, the length of the oligonucleotides, the G-C content of the oligonucleotides, and the expected degree of non-homology between the two oligonucleotides, if known. Methods of adjusting the stringency of annealing conditions are known (*see, e.g.*, Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York).

As used herein, the term "transgene" means an exogenous nucleic acid sequence which exogenous nucleic acid is encoded by a transgenic cell or mammal.

A "recombinant cell" is a cell that comprises a transgene. Such a cell may be a eukaryotic cell or a prokaryotic cell. Also, the transgenic cell encompasses, but is not limited to, an embryonic stem cell comprising the transgene, a cell obtained from a chimeric mammal derived from a transgenic ES cell where the cell comprises the transgene, a cell obtained from a transgenic

mammal, or fetal or placental tissue thereof, and a prokaryotic cell comprising the transgene.

By the term "exogenous nucleic acid" is meant that the nucleic acid has been introduced into a cell or an animal using technology which has been developed for the purpose of facilitating the introduction of a nucleic acid into a cell or an animal.

By "substrate binding arm" is meant that portion of a DNA enzyme which is complementary to (*i.e.*, able to base-pair with) a portion of its substrate. Generally, such complementary sequence is 100%, but can be less, *e.g.*, 80%, if desired. For example, as few as 10 bases out of 14 may be base-paired.

By "tag" polypeptide is meant any protein which, when linked by a peptide bond to a protein of interest, may be used to localize the protein, to purify it from a cell extract, to immobilize it for use in binding assays, or to otherwise study its biological properties and/or function.

As used herein, the term "transgenic mammal" means a mammal, the germ cells of which comprise an exogenous nucleic acid.

As used herein, to "treat" means reducing the frequency with which symptoms of the prostate cancer, are experienced by a patient.

By the term "vector" as used herein, is meant any plasmid or virus encoding an exogenous nucleic acid. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into virions or cells, such as, for example, polylysine compounds and the like. The vector may be a viral vector which is suitable as a delivery vehicle for delivery of the PCAM-1 protein or nucleic acid encoding a mammalian PCAM-1, or a ribozyme complementary to a nucleic acid encoding PCAM-1, or a portion thereof, to the patient, or the vector may be a non-viral vector which is suitable for the same purpose. Examples of viral and non-viral vectors for delivery of DNA to cells and tissues are well known in the art and are described, for example, in Ma et al. (1997, Proc. Natl. Acad. Sci. U.S.A. 94:12744-12746). Examples of viral vectors include, but are not limited to, a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus, a recombinant avian pox virus, and the like (Cranage et al., 1986, EMBO J. 5:3057-3063; International Patent Application No. WO94/17810, published August 18, 1994; International

Patent Application No. WO94/23744, published October 27, 1994). Examples of non-viral vectors include, but are not limited to, liposomes, polyamine derivatives of DNA, and the like.

A "knock-out targeting vector," as the term is used herein, means a vector comprising two nucleic acid sequences each of which is complementary to a nucleic acid regions flanking a target sequence of interest which is to be deleted and/or replaced by another nucleic acid sequence. The two nucleic acid sequences therefore flank the target sequence which is to be removed by the process of homologous recombination

Description.

I. Isolated nucleic acids

A. Sense nucleic acids

The present invention includes an isolated nucleic acid encoding a mammalian PCAM-1, or a fragment thereof, wherein the nucleic acid shares at least about 98% identity with a nucleic acid having the sequence SEQ ID NO:1. Preferably, the nucleic acid is about 99% homologous to SEQ ID NO:1. Even more preferably, the nucleic acid is SEQ ID NO:1.

In another aspect, the present invention includes an isolated nucleic acid encoding a mammalian PCAM-1, or a fragment thereof, wherein the protein encoded by the nucleic acid shares at least about 97%% homology with the amino acid sequence SEQ ID NO:2. Preferably, the nucleic acid is about 98% homologous, and most preferably, about 100% homologous to SEQ ID NO:2. Even more preferably, the PCAM-1 protein encoded by the nucleic acid is SEQ ID NO:2.

One skilled in the art would appreciate, based upon the disclosure provided herein, that mammalian PCAM-1 homologs likely exist and can be readily identified and isolated using the methods described herein using the sequence data disclosed herein. Thus, the present invention encompasses additional PCAM-1s, both human isoforms and PCAM-1 homologs from other species, that can be readily identified based upon the disclosure provided herein.

The isolated nucleic acid of the invention should be construed to include an RNA or a DNA sequence encoding a PCAM-1 protein of the invention, and any modified forms thereof, including chemical modifications of the DNA or

RNA which render the nucleotide sequence more stable when it is cell free or when it is associated with a cell. Chemical modifications of nucleotides may also be used to enhance the efficiency with which a nucleotide sequence is taken up by a cell or the efficiency with which it is expressed in a cell. Any and all combinations of modifications of the nucleotide sequences are contemplated in the present invention.

The present invention should not be construed as being limited solely to the nucleic and amino acid sequences disclosed herein. Once armed with the present invention, it is readily apparent to one skilled in the art that other nucleic acids encoding PCAM-1 proteins such as those present in other species of mammals (*e.g.*, ape, gibbon, bovine, ovine, equine, porcine, canine, feline, and the like) can be obtained by following the procedures described herein in the experimental details section for the isolation of human PCAM-1 nucleic acids encoding PCAM-1 polypeptides as disclosed herein (*e.g.*, screening of genomic or cDNA libraries), and procedures that are well-known in the art (*e.g.*, reverse transcription PCR using mRNA samples and antibody-based methods) or to be developed.

Further, any number of procedures may be used for the generation of mutant, derivative or variant forms of PCAM-1 using recombinant DNA methodology well known in the art such as, for example, that described in Sambrook et al. (1989, In: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York) and Ausubel et al. (1997, Current Protocols in Molecular Biology, Green & Wiley, New York).

Procedures for the introduction of amino acid changes in a protein or polypeptide by altering the DNA sequence encoding the polypeptide are well known in the art and are also described in Sambrook et al. (1989, *supra*); Ausubel et al. (1997, *supra*).

The invention includes a nucleic acid encoding a mammalian PCAM-1 wherein the nucleic acid encoding a tag polypeptide is covalently linked thereto. That is, the invention encompasses a chimeric nucleic acid wherein the nucleic acid sequences encoding a tag polypeptide is covalently linked to the nucleic acid encoding human PCAM-1. Such tag polypeptides are well known in the art and include, for instance, green fluorescent protein, myc, myc-pyruvate kinase (myc-PK), His_6, maltose biding protein (MBP), an influenza virus

hemagglutinin tag polypeptide, a flag tag polypeptide, and a glutathione-S-transferase (GST) tag polypeptide. However, the invention should in no way be construed to be limited to the nucleic acids encoding the above-listed tag polypeptides. Rather, any nucleic acid sequence encoding a polypeptide which may function in a manner substantially similar to these tag polypeptides should be construed to be included in the present invention.

The nucleic acid comprising a nucleic acid encoding a tag polypeptide can be used to localize PCAM-1 within a cell, a tissue, and/or a whole organism (*e.g.*, a mammalian embryo), detect PCAM-1 secreted from a cell, and to study the role(s) of PCAM-1 in a cell. Further, addition of a tag polypeptide facilitates isolation and purification of the "tagged" protein such that the proteins of the invention can be produced and purified readily.

The invention also includes a duplex (*i.e.*, double-stranded) nucleic acid that specifically binds with a mammalian PCAM-1 polypeptide. One skilled in the art would understand, based upon the disclosure provided herein, that such duplex nucleic acids include PCAM-1 probe 1 (5'- CACGGATG – 3' [SEQ ID NO:5]) and PCAM-1 probe 2 (5' - CACAATGA – 3' [SEQ ID NO:6]), 5'-CACAATG-3' (SEQ ID NO:7), and 5'-CACAATGTTTTTGT-3' (SEQ ID NO:8). The skilled artisan would appreciate that nucleic acids that specifically bind with PCAM-1 can be used to detect the presence or absence of PCAM-1 in a protein sample derived from solid tissue or fluids, and to assess the level of PCAM-1 therein, as more fully discussed elsewhere herein. Thus, the duplex (*i.e.*, double-stranded, which is used interchangeably herein), nucleic acids are powerful probes useful for detection of any disease, disorder, or condition associated with mal-expression of PCAM-1, including, but not limited to, prostate cancer.

B. Antisense nucleic acids

In certain situations, it may be desirable to inhibit expression of PCAM-1 and the invention therefore includes compositions useful for inhibition of PCAM-1 expression. Thus, the invention features an isolated nucleic acid complementary to a portion or all of a nucleic acid encoding a mammalian PCAM-1 which nucleic acid is in an antisense orientation with respect to transcription. Preferably, the antisense nucleic acid is complementary with a nucleic acid having

at least about 95% homology with SEQ ID NO:1. Preferably, the nucleic acid is about 96% homologous, more preferably, about 97% homologous, more preferably, about 98% homologous, and most preferably, about 99% homologous to a nucleic acid complementary to a portion or all of a nucleic acid encoding a mammalian PCAM-1 having the sequence SEQ ID NO:1, or a fragment thereof, which is in an antisense orientation with respect to transcription. Most preferably, the nucleic acid is complementary to a portion or all of a nucleic acid having the sequence SEQ ID NO:1, or a fragment thereof. Such antisense nucleic acid serves to inhibit the expression, function, or both, of a PCAM-1.

Alternatively, antisense molecules of the invention may be made synthetically and then provided to the cell. Antisense oligomers of between about 10 to about 30, and more preferably about 15 nucleotides, are preferred, since they are easily synthesized and introduced into a target cell. Synthetic antisense molecules contemplated by the invention include oligonucleotide derivatives known in the art which have improved biological activity compared to unmodified oligonucleotides (*see* Cohen, *supra*; Tullis, 1991, U.S. Patent No. 5,023,243, incorporated by reference herein in its entirety).

II. Isolated polypeptides

The invention also includes an isolated polypeptide comprising a mammalian PCAM-1 molecule. Preferably, the isolated polypeptide is about 97% homologous, even more preferably, about 98% homologous, and most preferably, about 99% homologous to SEQ ID NO:2. More preferably, the isolated polypeptide comprising a mammalian PCAM-1 is human PCAM-1. Most preferably, the isolated polypeptide comprising a mammalian PCAM-1 is SEQ ID NO:2.

The present invention also provides for analogs of proteins or peptides which comprise a PCAM-1 as disclosed herein. Analogs may differ from naturally occurring proteins or peptides by conservative amino acid sequence differences or by modifications which do not affect sequence, or by both. For example, conservative amino acid changes may be made, which although they alter the primary sequence of the protein or peptide, do not normally alter its function.

Conservative amino acid substitutions typically include substitutions within the following groups:

glycine, alanine;
valine, isoleucine, leucine;
aspartic acid, glutamic acid;
asparagine, glutamine;
serine, threonine;
lysine, arginine;
phenylalanine, tyrosine.

Modifications (which do not normally alter primary sequence) include *in vivo*, or *in vitro*, chemical derivatization of polypeptides, *e.g.*, acetylation, or carboxylation. Also included are modifications of glycosylation, *e.g.*, those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; *e.g.*, by exposing the polypeptide to enzymes which affect glycosylation, *e.g.*, mammalian glycosylating or deglycosylating enzymes. Also embraced are sequences which have phosphorylated amino acid residues, *e.g.*, phosphotyrosine, phosphoserine, or phosphothreonine.

Also included are polypeptides which have been modified using ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent. Analogs of such polypeptides include those containing residues other than naturally occurring L-amino acids, *e.g.*, D-amino acids or non-naturally occurring synthetic amino acids. The peptides of the invention are not limited to products of any of the specific exemplary processes listed herein.

The present invention should also be construed to encompass "mutants," "derivatives," and "variants" of the peptides of the invention (or of the DNA encoding the same) which mutants, derivatives and variants are PCAM-1 peptides which are altered in one or more amino acids (or, when referring to the nucleotide sequence encoding the same, are altered in one or more base pairs) such that the resulting peptide (or DNA) is not identical to the sequences recited herein, but has the same biological property as the peptides disclosed herein, in that the

peptide has biological/biochemical properties of the PCAM-1 peptide of the present invention.

A biological property of a PCAM-1 protein should be construed but not be limited to include, the ability to specifically bind with a nucleic acid sequence having the sequence of at least one of CACGGATG (PCAM-1 probe 1; SEQ ID NO:5) and CACAATGA (PCAM-1 probe 2; SEQ ID NO:6), CACAATG (SEQ ID NO:7), and CACAATGTTTTTGT (SEQ ID NO:8), and the like.

Further, the invention should be construed to include naturally occurring variants or recombinantly derived mutants of PCAM-1 sequences, which variants or mutants render the protein encoded thereby either more, less, or just as biologically active as the full-length clones of the invention.

The nucleic acids, and peptides encoded thereby, are useful tools for elucidating the function(s) of PCAM-1 in a cell. Further, nucleic and amino acids comprising mammalian PCAM-1 are useful diagnostics which can be used, for example, to identify a compound that affects PCAM-1 expression and is a potential prostate anticancer anti-cell proliferation drug candidate. The nucleic acids, the proteins encoded thereby, or both, can be administered to a mammal to increase or decrease expression of PCAM-1 in the mammal. This can be beneficial for the mammal in situations where under or over-expression of PCAM-1 in the mammal mediates a disease or condition associated with altered expression of PCAM-1 compared with normal expression of PCAM-1 in a healthy mammal.

Additionally, the nucleic and amino acids of the invention can be used to produce recombinant cells and transgenic non-human mammals, which are useful tools for the study of PCAM-1 action, the identification of novel diagnostics and therapeutics for treatment of prostate cancer, and possibly other cancers, and for elucidating the cellular role(s) of PCAM-1, among other things.

Further, the nucleic and amino acids of the invention can be used diagnostically, either by assessing the level of gene expression or protein expression, to assess severity, stage and prognosis of prostate tumors and the like. The nucleic acids and proteins of the invention are also useful in the development of assays to assess the efficacy of a treatment for prostate tumors. That is, the nucleic acids and polypeptides of the invention can be used to detect the effect of

various therapies on PCAM-1 expression, thereby ascertaining the effectiveness of the therapies.

III. Vectors

In other related aspects, the invention includes an isolated nucleic acid encoding a mammalian PCAM-1 operably linked to a nucleic acid comprising a promoter/regulatory sequence such that the nucleic acid is preferably capable of directing expression of the protein encoded by the nucleic acid. Thus, the invention encompasses expression vectors and methods for the introduction of exogenous DNA into cells with concomitant expression of the exogenous DNA in the cells such as those described, for example, in Sambrook et al. (1989, *supra*), and Ausubel et al. (1997, *supra*).

Expression of PCAM-1, an antisense nucleic acid complementary to a nucleic acid encoding PCAM-1, and/or a ribozyme complementary to a nucleic acid encoding PCAM-1, either alone or fused to a detectable tag polypeptide, in cells which either do not normally express the PCAM-1, antisense PCAM-1, or PCAM-1 ribozyme, or which do not express such PCAM-1 constructs fused with a tag polypeptide, may be accomplished by generating a plasmid, viral, or other type of vector comprising the desired nucleic acid operably linked to a promoter/regulatory sequence which serves to drive expression of the protein, with or without tag, in cells in which the vector is introduced.

Many promoter/regulatory sequences useful for driving constitutive expression of a gene are available in the art and include, but are not limited to, for example, the adenovirus (ADV), the cytomegalovirus (CMV) immediate early promoter enhancer sequence, the SV40 early promoter, both of which were used in the experiments disclosed herein, as well as the Rous sarcoma virus promoter, and the like. Moreover, inducible and tissue specific expression of the nucleic acid encoding PCAM-1 may be accomplished by placing the nucleic acid encoding PCAM-1, with or without a tag, under the control of an inducible or tissue specific promoter/regulatory sequence. Examples of tissue specific or inducible promoter/regulatory sequences which are useful for his purpose include, but are not limited to the MMTV LTR inducible promoter, and the SV40 late enhancer/promoter. In addition, promoters which are well known in the art which

are induced in response to inducing agents such as metals, glucocorticoids, and the like, are also contemplated in the invention. Thus, it will be appreciated that the invention includes the use of any promoter/regulatory sequence, which is either known or unknown, and which is capable of driving expression of the desired protein operably linked thereto.

Expressing PCAM-1 using a vector allows the isolation of large amounts of recombinantly produced protein. Further, where the lack or decreased level of PCAM-1 expression causes a disease, disorder, or condition associated with such expression, the expression of PCAM-1 driven by a promoter/regulatory sequence can provide useful therapeutics including, but not limited to, gene therapy whereby PCAM-1 is provided. A disease, disorder or condition associated with a decreased level of expression, level of protein, or decreased activity of the protein, for which administration of PCAM-1 can be useful can includes, but is not limited to, prostate cancer, and other cancers, and the like. Therefore, the invention includes not only methods of inhibiting PCAM-1 expression, translation, and/or activity, but it also includes methods relating to increasing PCAM-1 expression, protein level, and/or activity since both decreasing and increasing PCAM-1 expression and/or activity can be useful in providing effective therapeutics.

Selection of any particular plasmid vector or other DNA vector is not a limiting factor in this invention and a wide plethora vectors is well-known in the art. Further, it is well within the skill of the artisan to choose particular promoter/regulatory sequences and to operably link those promoter/regulatory sequences to a DNA sequence encoding a desired polypeptide. Such technology is well known in the art and is described, for example, in Sambrook et al. (1989, In: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York) and Ausubel et al. (1997, Current Protocols in Molecular Biology, Green & Wiley, New York).

The invention thus includes a vector comprising an isolated nucleic acid encoding a mammalian PCAM-1. The incorporation of a desired nucleic acid into a vector and the choice of vectors is well-known in the art as described in, for example, Sambrook et al., *supra*, and Ausubel et al., *supra*.

The invention also includes cells, viruses, proviruses, and the like, containing such vectors. Methods for producing cells comprising vectors and/or

exogenous nucleic acids are well-known in the art. *See, e.g.*, Sambrook et al., *supra*; Ausubel et al., *supra*.

The nucleic acids encoding PCAM-1 may be cloned into various plasmid vectors. However, the present invention should not be construed to be limited to plasmids or to any particular vector. Instead, the present invention should be construed to encompass a wide plethora of vectors which are readily available and/or well-known in the art.

IV. Antisense molecules and ribozymes

Further, the invention includes a recombinant cell comprising an antisense nucleic acid which cell is a useful model for elucidating the role(s) of PCAM-1 in cellular processes. That is, without wishing to be bound by any particular theory, the increased expression of PCAM-1 in prostate cancer tissues but not in benign prostate tumors or in normal prostate tissues indicates that PCAM-1 is involved in cell survival and cell proliferation associated with tumor growth. Accordingly, a transgenic cell comprising an antisense nucleic acid complementary to PCAM-1 is a useful tool for the study of the mechanism(s) of action of PCAM-1 and its role(s) in the cell and for the identification of therapeutics that ameliorate the effect(s) of PCAM-1 over-expression. Further, methods of decreasing PCAM-1 expression and/or activity in a cell can provide useful diagnostics and/or therapeutics for diseases, disorders or conditions mediated by or associated with increased PCAM-1 expression, increased level of PCAM-1 protein in a cell or secretion therefrom, and/or increased PCAM-1 activity. Such diseases, disorders or conditions include, but are not limited to, prostate cancer, and the like.

One skilled in the art will appreciate that one way to decrease the levels of PCAM-1 mRNA and/or protein in a cell is to inhibit expression of the nucleic acid encoding the protein. Expression of PCAM-1 may be inhibited using, for example, antisense molecules, and also by using ribozymes or double-stranded RNA as described in, for example, Wianny and Kernicka-Goetz (2000, Nature Cell Biol. 2:70-75).

A. Antisense molecules

Antisense molecules and their use for inhibiting gene expression are well known in the art (*see, e.g.*, Cohen, 1989, In: Oligodeoxyribonucleotides,

Antisense Inhibitors of Gene Expression, CRC Press). Antisense nucleic acids are DNA or RNA molecules that are complementary, as that term is defined elsewhere herein, to at least a portion of a specific mRNA molecule (Weintraub, 1990, Scientific American 262:40). In the cell, antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule thereby inhibiting the translation of genes.

The use of antisense methods to inhibit the translation of genes is known in the art, and is described, for example, in Marcus-Sakura (1988, Anal. Biochem. 172:289). Such antisense molecules may be provided to the cell via genetic expression using DNA encoding the antisense molecule as taught by Inoue (1993, U.S. Patent No. 5,190,931).

Alternatively, antisense molecules of the invention may be made synthetically and then provided to the cell. Antisense oligomers of between about 10 to about 100, and more preferably about 15 to about 50 nucleotides, are preferred, since they are easily synthesized and introduced into a target cell. Synthetic antisense molecules contemplated by the invention include oligonucleotide derivatives known in the art which have improved biological activity compared to unmodified oligonucleotides (*see* Cohen, *supra*; Tullis, 1991, U.S. Patent No. 5,023,243, incorporated by reference herein in its entirety).

B. Ribozymes

Ribozymes and their use for inhibiting gene expression are also well known in the art (*see, e.g.*, Cech et al., 1992, J. Biol. Chem. 267:17479-17482; Hampel et al., 1989, Biochemistry 28:4929-4933; Eckstein et al., International Publication No. WO 92/07065; Altman et al., U.S. Patent No. 5,168,053, incorporated by reference herein in its entirety). Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences encoding these RNAs, molecules can be engineered to recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, 1988, J. Amer. Med. Assn. 260:3030). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes, namely, tetrahymena-type (Hasselhoff, 1988, Nature 334:585) and hammerhead-type. Tetrahymena-type ribozymes recognize sequences which are four bases in length, while hammerhead-type ribozymes recognize base sequences 11-18 bases in length. The longer the sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to tetrahymena-type ribozymes for inactivating specific mRNA species, and 18-base recognition sequences are preferable to shorter recognition sequences which may occur randomly within various unrelated mRNA molecules.

Ribozymes useful for inhibiting the expression of PCAM-1 may be designed by incorporating target sequences into the basic ribozyme structure which are complementary to the mRNA sequence of the PCAM-1 encoded by PCAM-1 or having at least about 80% homology to at least one of SEQ ID NO:1 and SEQ ID NO:3. Ribozymes targeting PCAM-1 can be synthesized using commercially available reagents (Applied Biosystems, Inc., Foster City, CA) or they may be genetically expressed from DNA encoding them.

This invention relates to DNA enzymes, or enzymatic nucleic acid molecules, directed to cleave RNA species that are required for cellular growth responses. In particular, the applicant describes the selection and function of DNA enzymes capable of cleaving RNA encoded by the PCAM-1 gene. Such DNA enzymes may be used to inhibit the survival of tumor cells in one or more cancers.

In the present invention, DNA enzymes that cleave PCAM-1 RNA are described *(i.e.*, PCAM-1 DNA ZYM-1). Those of ordinary skill in the art will understand that from the examples described that other DNA enzymes that cleave target RNAs required for cell proliferation may be readily designed and are within the invention. Such RNAs may have at least 90% homology to PCAM-1 in humans.

Six basic varieties of naturally-occurring enzymatic RNAs (*e.g.*, hepatitis, delta viruses, group I intron, group II intron, Rnase PRNA, and Neurospora VSRNA) are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions.

In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic

nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a DNA enzyme is advantageous over other technologies, since the concentration of DNA enzyme necessary to effect a therapeutic treatment is lower. This advantage reflects the ability of the DNA enzyme to act enzymatically. Thus, a single DNA enzyme molecule is able to cleave many molecules of target RNA. In addition, the DNA enzyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base-pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can be chosen to completely eliminate catalytic activity of a DNA enzyme. One other advantage of a DNA enzyme is that the "half-life" *in vivo* is days rather than hours reported for ribozymes or anti-sense sequences.

Nucleic acid molecules having an endonuclease enzymatic activity are able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence-specific manner. Such enzymatic DNA or RNA molecules can be targeted to virtually any RNA transcript, and efficient cleavage achieved in vitro (Zaug et al., 1986, Nature 324:429; Uhlenbeck, 1987, Nature 328:596; Kim et al., 1987, Proc. Natl. Acad. Sci. USA 84:8788; Dreyfus, 1988, Einstein Quart. J. Bio. Med., 6:92; Haseloff and Gerlach, 1988, Nature 334:585; Cech, 1988, J. Amer. Med. Assn. 260:3030; and Jefferies et al., 1989, Nucleic Acids Res. 17:1371).

Because of their sequence-specificity, trans-cleaving DNA enzymes show promise as therapeutic agents for human disease. DNA enzymes can be designed to cleave specific RNA targets within the background of cellular RNA. Such a cleavage event renders the RNA non-functional and abrogates protein expression from that RNA. In this manner, synthesis of a protein associated with a disease state can be selectively inhibited.

DNA enzymes that cleave the specified sites in PCAM-1 RNAs (*i.e.*, PCAM-1 DNA ZYM-1) represent a novel therapeutic approach to treat diseases, such as cancer and other conditions. The data disclosed elsewhere herein demonstrates that PCAM-1 DNA ZYMs can and do inhibit the activity of PCAM-1 and that the catalytic activity of the PCAM-1 DNA ZYM-1 is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other PCAM-1 DNA ZYMs that cleave these sites in PCAM-1 RNAs can be readily designed and are within the scope of this invention.

In one of the preferred embodiments of the inventions herein, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif. Examples of such hammerhead motifs are described by Dreyfus, *supra*, Rossi et al., 1992, AIDS Research and Human Retroviruses 8:183; of hairpin motifs by Hampel et al., EP0360257, Hampel and Tritz, 1989, Biochemistry 28:4929, Feldstein et al., 1989, Gene 82:53, Haseloff and Gerlach, 1989, Gene 82:43; and Hampel et al., 1990, Nucleic Acids Res. 18:299. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule (or multiple fragments of such molecules) of this invention is that is has a specific substrate binding site or arm(s) which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule (enzymatic portion).

Such arms are shown generally in Figures 3 and 4 as discussed below. That is, these arms contain sequences within a DNA enzyme which are intended to bring DNA enzyme and target RNA together through complementary base-pairing interactions; e.g., DNA enzyme sequences with stems I and II (*i.e.*, 2 base pair) of a standard hammerhead DNA enzyme make up the substrate-binding domain (see Figure 4).

In a preferred embodiment the invention provides a method for producing a class of enzymatic cleaving agents (*i.e.*, PCAM-1 DNA ZYMs) which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNA encoding PCAM-1 proteins such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic

acids. That is, one skilled in the art would appreciate, based upon the disclosure provided herein, that enzymatic nucleic acids can be used in combination with each other, and also in combination with other compounds including, but not limited to, chemotherapeutic agents, small molecules, peptidomimetics, antisense nucleic acids, antibodies, and the like. Thus, the invention is not limited to using a single enzymatic nucleic acid by itself; rather, the invention also encompasses using a ribozyme in combination and/or in concert with another compound, including ribozymes directed against other mRNA molecules encoding proteins other than PCAM-1. Such other ribozymes can be directed against proteins such as, but not limited to, MMP-2 and VEGF-1, and the like.

An enzymatic nucleic acid molecule can be delivered exogenously to specific cells or tissues as required. Alternatively, the PCAM-1 DNA ZYMs can be expressed from DNA/RNA vectors that are delivered to specific cells or tissues.

Synthesis of nucleic acids greater that 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs (*e.g.*, antisense oligonucleotides, hammerhead or the hairpin DNA enzymes) are used for exogenous delivery (*i.e.*, about 30 to 40 in length). Preferably, the enzymatic nucleic acid is from about 10 to 100 nucleotides, even more preferably, about 20 to 80 nucleotides, yet more preferably, from about 25 to 60 nucleotides, and most preferably, from about 30 to 50 nucleotides in length. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of the mRNA structure.

However, these nucleic acid molecules can also be expressed within cells from eukaryotic promoters (*e.g.*, Izant and Weintraub, 1985, Science 229:345; McGarry and Lindquist, 1986, Proc. Natl. Acad. Sci. USA 83:399; Sullenger Scanlon et al., 1991, Proc. Natl. Acad. Sci. USA 88:10591-10595; Kashani-Sabet et al., 1992, Antisense Res. Dev. 2:3-15; Dropulic et al., 1992, J. Virol. 66:1432-1441; Weerasinghe et al., 1991, J. Virol. 65:5531-5534; Ojwang et al., 1992, Proc. Natl. Acad. Sci. USA 89:10802-10806; Chen et al., 1992, Nucleic Acids Res. 20:4581-4589; Sarver et al., 1990, Science 247:1222-1225; Thompson et al., 1995, Nucleic Acids Res. 23:2259). Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity

of such nucleic acids can be augmented by their release from the primary transcript by a DNA enzyme or ribozyme (Draper et al., PCT WO93/23569, and Sullivan et al., PCT WO94/02595, both hereby incorporated in their totality by reference herein; Ohkawa et al., 1992, Nucleic Acids Symp. Ser. 27:15-16; Taira et al., 1991, Nucleic Acids Res. 19:5125-5130; Ventura et al., 1993, Nucleic Acids Res. 21:3249-3255; Chowrira et al., 1994, J. Biol. Chem. 269:25856).

Such DNA enzymes or PCAM-1 DNA ZYMs are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the levels of PCAM-1 activity in a diseased cell or tissue.

PCAM-1 DNA ZYMs are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the PCAM-1 DNA ZYMs have binding arms which are complementary to the sequences in Figure 1.

Thus, in a first aspect, the invention features PCAM-1 DNA ZYMs that inhibit gene expression and/or cell proliferation via cleavage of RNA expressed from the PCAM-1 gene. These chemically or enzymatically synthesized RNA molecules contain substrate binding domains, *i.e.*, "binding arms," that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably PCAM-1 DNA ZYMs of the hammerhead or hairpin motif. Upon binding, the PCAM-1 DNA ZYMs cleave the target mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, cell proliferation and/or survival is inhibited.

In a preferred embodiment, the PCAM-1 DNA ZYMs cleave PCAM-1 mRNA and inhibit cell proliferation and/or survival. Such PCAM-1 DNA ZYMs are useful for the prevention and/or treatment of cancer or other diseases. PCAM-1 DNA ZYMs are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to smooth muscle cells. The RNA or RNA complexes can be locally administered to relevant tissues through the use of a catheter, infusion pump or stent, with or without their incorporation in

biopolymers. The PCAM-1 DNA ZYMs, similarly delivered, also are useful for inhibiting proliferation and/or survival of certain cancers associated with elevated levels of the PCAM-1, particularly prostate cancer. Using the methods described herein, other PCAM-1 DNA ZYMs that cleave PCAM-1 and thereby inhibit tumor cell proliferation and/or survival may be derived and used as described above.

In another aspect of the invention, PCAM-1 DNA ZYMs that cleave target molecules and inhibit PCAM-1 activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. DNA enzyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, cytomegalovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the PCAM-1 DNA ZYMs are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of PCAM-1 DNA ZYMs. Such vectors might be repeatedly administered as necessary. Once expressed, the PCAM-1 DNA ZYMs cleave the target mRNA. Delivery of PCAM-1 DNA ZYM 1 expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell such as intercalating the PCAM-1 DNA ZYM-1 in a polymer matrix which enables slow delivery of the enzyme to cells (for a review see Couture and Stinchcomb, 1986, T.I.G. 12:510). The present invention encompasses these and other methods known in the art for delivery and expression of ribozymes, as well as methods developed in the future.

These PCAM-1 DNA ZYMs, individually, or in combination or in conjunction with other drugs can be used to treat diseases or conditions discussed above. For example, to treat a disease or condition associated with PCAM-1 levels, the patient may be treated, or other appropriate cells may be treated, as is evident to those skilled in the art.

In a further embodiment, the described PCAM-1 DNA ZYMs can be used in combination with other known treatments or surgical procedures (*e.g.*, cryoablation), to treat conditions or diseases discussed above. For example, the described PCAM-1 DNA ZYMs could be used in combination with one of more

known therapeutic agents to treat cancer.

Targets

Hammerhead or hairpin PCAM-1 DNA ZYMs were designed that could bind and were individually analyzed by computer folding (Jaeger et al., 1989, Proc. Natl. Acad. Sci. USA 86:7706) to assess whether the PCAM-1 DNA ZYMs sequences fold into the appropriate secondary structure. Those PCAM-1 DNA ZYMs with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

PCAM-1 DNA ZYMs of the hammerhead or hairpin motif are designed to anneal to the initial 5' region of the mRNA message (*e.g.*, basepairs 1-41). The binding arms are complementary to the target site sequences described above. The PCAM-1 DNA ZYMs were chemically synthesized. The method of synthesis used follows the procedure for normal oligonucleotide synthesis as described in Usman et al. (1987, J. Am. Chem. Soc. 109:7845), Scaringe et al. (1990, Nucleic Acids Res. 18:5433), and Wincott et al. (1995, Nucleic Acids Res. 23:2677-2684), and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end.

One skilled in the art would understand, based upon the disclosure provided herein, that once armed with the sequence of a nucleic acid encoding PCAM-1 (*e.g.*, a nucleic acid sharing greater than about 98% sequence identity with SEQ ID NO:1), it would be routine for the skilled artisan to produce various ribozymes that specifically cleave an mRNA encoding a PCAM-1 polypeptide. That is, by selecting various 30-40 nucleotide sequences along the mRNA sequence assaying the putative ribozyme for PCAM-1 mRNA cleaving activity as disclosed herein, or as known in the art or as developed in the future, various PCAM-1 specific enzymatic nucleic acids can be identified and produced. Therefore, such enzymatic nucleic acids that specifically cleave PCAM-1 mRNA are encompassed in the present invention. Such enzymes include, but are not limited to, those exemplified herein having the sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), sequence

AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11). The skilled artisan would understand, based upon the disclosure provided herein, that these ribozymes are merely exemplary and that the invention is in no way limited to these sequences.

Hairpin PCAM-1 DNA ZYMs can be synthesized in two parts and annealed to reconstruct the active DNA enzyme (Chowrira and Burke, 1992, Nucleic Acids Res. 20:2835-2840). PCAM-1 DNA ZYMs can also be synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, Methods Enzymol. 180:51).

PCAM-1 DNA ZYMs can be purified by gel electrophoresis using general methods or purified by high pressure liquid chromatography (HPLC; see Wincott et al., *supra*) the totality of which is hereby incorporated herein by reference) and are resuspended in water.

The general structure of the PCAM-1 DNA ZYMs that are chemically synthesized, useful in this study, is depicted in Figures 3 and 4. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the DNA enzyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead PCAM-1 DNA ZYMs can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form.

Optimizing PCAM-1 DNA ZYMs DNA enzyme Activity

DNA enzyme activity can be optimized as described by Draper et al., *supra*. The details will not be repeated here, but include altering the length of the DNA enzyme binding arms (stems I and III, see Figure 4), or chemically synthesizing PCAM-1 DNA ZYMs with modifications (base, sugar and/or phosphate) that prevent their degradation by serum ribonucleases and/or enhance their enzymatic activity (*see, e.g.*, Eckstein et. al., International Publication No. WO 92/07065; Perrault et al., 1990, Nature 344:565; Pieken et al., 1991, Science 253:314; Usman and Cedergren, 1992, Trends in Biochem. Sci. 17:334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International

Publication No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; and Burgin et al., supra. All of these studies describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of enzymatic RNA molecules). Modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements are desired. (All of these publications are hereby incorporated by reference herein).

The enzymatic nucleic acid having chemical modifications which maintain or enhances enzymatic activity is provided. Such nucleic acid is also generally more resistant to nucleases than unmodified nucleic acid. By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and thymidine at 1' position or their equivalents; such bases may be used within the catalytic core of the enzyme as well as in the substrate-binding regions. As noted above, substitution in the core may decrease in vitro activity but enhances stability. Thus, in a cell and/or *in vivo* the activity may not be significantly lowered. As exemplified herein such DNA enzymes are useful in a cell and/or *in vivo* even if activity over all is reduced 10 fold. Such DNA enzymes herein are said to "maintain" the enzymatic activity on all PCAM-1 DNA ZYMs.

Sullivan, et al., *supra*, describes the general methods for delivery of enzymatic RNA molecules. PCAM-1 DNA ZYMs may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by ionophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules or polymer matrices, and bioadhesive microspheres. For some indications, PCAM-1 DNA ZYMs may be directly delivered ex vivo to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of DNA enzyme delivery and administration are provided in Sullivan et al., *supra*, and Draper et al., *supra*, which have been incorporated by reference herein.

Another means of accumulating high concentrations of a PCAM-1

DNA ZYM(s) within cells is to incorporate the PCAM-1 DNA ZYM-encoding sequences into a DNA or RNA expression vector. Transcription of the PCAM-1 DNA ZYM sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, and the like) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, Proc. Natl. Acad. Sci. USA 87:6743-6747; Gao and Huang, 1993, Nucleic Acids Res. 21:2867-2872; Lieber et al., 1993, Methods Enzymol. 217:47-66; Zhou et al., 1990, Mol. Cell. Biol. 10:4529-4537).

Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (*e.g.*, Kashani-Sabet et al., 1992, Antisense Res. Dev. 2:3-15; Ojwang et al., 1992, Proc. Natl. Acad. Sci. USA 89:10802-10806; Chen et al., 1992, Nucleic Acids Res. 20:4581-4589; Yu et al., 1993, Proc. Natl. Acad. Sci. USA 90:6340-6344; L'Huillier et al., 1992, EMBO J. 11:4411-4418; Lisziewicz et al., 1993, Proc. Natl. Acad. Sci. USA. 90:8000-8004; Thompson et al., 1995, Nucleic Acids Res. 23:2259; Sullenger & Cech, 1993, Science 262:1566). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as cytomegalovirus, adenovirus or adeno-associated virus vectors, and the like), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, *supra*).

In a preferred embodiment of the invention, a transcription unit expressing a PCAM-1 DNA ZYM-1 that cleaves mRNAs encoded by PCAM-1 is inserted into a plasmid DNA vector of an adenovirus or adeno-associated virus DNA viral vector or a retroviral RNA vector. Viral vectors have been used to transfer genes and lead to either transient or long term gene expression (Zabner et al., 1993 Cell 75, 207; Carter, 1992 Curr. Opi. Biotech. 3:533). The adenovirus vector is delivered as recombinant adenoviral particles. The DNA may be delivered alone or complexed with vehicles (as described for RNA above). The recombinant

adenovirus or AAV particles are locally administered to the site of treatment, *e.g.*, through incubation or inhalation *in vivo* or by direct application to cells or tissues *ex vivo*. Retroviral vectors have also been used to express ribozymes in mammalian cells (Ojwang et al., 1992, supra; Thompson et al., 1995, supra; Couture and Stinchcomb, 1996; supra). In another preferred embodiment, the PCAM-1 DNA ZYMs is administered to the site of PCAM-1 expression (*e.g.*, tumor cells) in an appropriate liposomal vesicle.

V. Recombinant cells and transgenic non-human mammals

The invention includes a recombinant cell comprising , *inter alia*, an isolated nucleic acid encoding PCAM-1, an antisense nucleic acid complementary thereto, a ribozyme complementary to the nucleic acid encoding PCAM-1, a nucleic acid encoding an antibody that specifically binds PCAM-1, and the like. In one aspect, the recombinant cell can be transiently transfected with a plasmid encoding a portion of the nucleic acid encoding PCAM-1. The nucleic acid need not be integrated into the cell genome nor does it need to be expressed in the cell. Moreover, the cell may be a prokaryotic or a eukaryotic cell and the invention should not be construed to be limited to any particular cell line or cell type. Such cells include, but are not limited to, fibroblasts, hepatocytes, skeletal muscle cells, and adipocytes.

In one aspect, the recombinant cell comprising an isolated nucleic acid encoding mammalian PCAM-1 is used to produce a transgenic non-human mammal. That is, the exogenous nucleic acid, or transgene as it is also referred to herein, of the invention is introduced into a cell, and the cell is then used to generate the non-human transgenic mammal. The cell into which the transgene is introduced is preferably an embryonic stem (ES) cell. However, the invention should not be construed to be limited solely to ES cells comprising the transgene of the invention nor to cells used to produce transgenic animals. Rather, a transgenic cell of the invention includes, but is not limited to, any cell derived from a transgenic animal comprising a transgene, a cell comprising the transgene derived from a chimeric animal derived from the transgenic ES cell, and any other comprising the transgene which may or may not be used to generate a non-human transgenic mammal.

Further, it is important to note that the purpose of transgene-comprising, *i.e.*, recombinant, cells should not be construed to be limited to the generation of transgenic mammals. Rather, the invention should be construed to include any cell type into which a nucleic acid encoding a mammalian PCAM-1 is introduced, including, without limitation, a prokaryotic cell and a eukaryotic cell comprising an isolated nucleic acid encoding mammalian PCAM-1.

When the cell is a eukaryotic cell, the cell may be any eukaryotic cell which, when the transgene of the invention is introduced therein, and the protein encoded by the desired gene is no longer expressed therefrom, a benefit is obtained. Such a benefit may include the fact that there has been provided a system in which lack of expression of the desired gene can be studied *in vitro* in the laboratory or in a mammal in which the cell resides, a system wherein cells comprising the introduced gene deletion can be used as research, diagnostic and therapeutic tools, and a system wherein animal models are generated which are useful for the development of new diagnostic and therapeutic tools for selected disease states in a mammal including, for example, prostate cancer, and the like.

Alternatively, the invention includes a eukaryotic cell which, when the transgene of the invention is introduced therein, and the protein encoded by the desired gene is expressed therefrom where it was not previously present or expressed in the cell or where it is now expressed at a level or under circumstances different than that before the transgene was introduced, a benefit is obtained. Such a benefit may include the fact that there has been provided a system in the expression of the desired gene can be studied *in vitro* in the laboratory or in a mammal in which the cell resides, a system wherein cells comprising the introduced gene can be used as research, diagnostic and therapeutic tools, and a system wherein animal models are generated which are useful for the development of new diagnostic and therapeutic tools for selected disease states in a mammal.

Such cell expressing an isolated nucleic acid encoding PCAM-1 can be used to provide PCAM-1 to a cell, tissue, or whole animal where a higher level of PCAM-1 can be useful to treat or alleviate a disease, disorder or condition associated with low level of PCAM-1 expression and/or activity. Such diseases, disorders or conditions can include, but are not limited to prostate cancer, and possibly other solid cancers or leukemias, AIDS, HIV infection , immune disorders

and inflammatory or degenerative disorders, and the like. Therefore, the invention includes a cell expressing PCAM-1 to increase or induce PCAM-1 expression, translation, and/or activity, where increasing PCAM-1 expression, protein level, and/or activity can be useful to treat or alleviate a disease, disorder or condition.

One of ordinary skill would appreciate, based upon the disclosure provided herein, that a "knock-in" or "knock-out" vector of the invention comprises at least two sequences homologous to two portions of the nucleic acid which is to be replaced or deleted, respectively. The two sequences are homologous with sequences that flank the gene; that is, one sequence is homologous with a region at or near the 5' portion of the coding sequence of the nucleic acid encoding PCAM-1 and the other sequence is further downstream from the first. One skilled in the art would appreciate, based upon the disclosure provided herein, that the present invention is not limited to any specific flanking nucleic acid sequences. Instead, the targeting vector may comprise two sequences which remove some or all (*i.e.*, a "knock-out" vector) or which insert (*i.e.*, a "knock-in" vector) a nucleic acid encoding PCAM-1, or a fragment thereof, from or into a mammalian genome, respectively. The crucial feature of the targeting vector is that it comprise sufficient portions of two sequences located towards opposite, *i.e.*, 5' and 3', ends of the PCAM-1 open reading frame (ORF) in the case of a "knock-out" vector, to allow deletion/insertion by homologous recombination to occur such that all or a portion of the nucleic acid encoding PCAM-1 is deleted from or inserted into a location on a mammalian chromosome.

The design of transgenes and knock-in and knock-out targeting vectors is well-known in the art and is described in standard treatises such as Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York), and the like. The upstream and downstream portions flanking or within the PCAM-1 coding region to be used in the targeting vector may be easily selected based upon known methods and following the teachings disclosed herein based on the disclosure provided herein including the nucleic and amino acid sequences of both mouse and human PCAM-1. Armed with these sequences, one of ordinary skill in the art would be able to construct the transgenes and knock-out vectors of the invention.

The invention further includes a knock-out targeting vector comprising a nucleic acid encoding a selectable marker such as, for example, a nucleic acid encoding the *neo*^{R} gene thereby allowing the selection of transgenic a cell where the nucleic acid encoding PCAM-1, or a portion thereof, has been deleted and replaced with the neomycin resistance gene by the cell's ability to grow in the presence of G418. However, the present invention should not be construed to be limited to neomycin resistance as a selectable marker. Rather, other selectable markers well-known in the art may be used in the knock-out targeting vector to allow selection of recombinant cells where the PCAM-1 gene has been deleted and/or inactivated and replaced by the nucleic acid encoding the selectable marker of choice. Methods of selecting and incorporating a selectable marker into a vector are well-known in the art and are describe in, for example, Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York).

As noted herein, the invention includes a non-human transgenic mammal comprising an exogenous nucleic acid inserted into a desired site in the genome thereof thereby deleting the coding region of a desired endogenous target gene, *i.e.*, a knock-out transgenic mammal. Further, the invention includes a transgenic non-human mammal wherein an exogenous nucleic acid encoding PCAM-1 is inserted into a site the genome, *i.e.*, a "knock-in" transgenic mammal. The knock-in transgene inserted may comprise various nucleic acids encoding, for example, a tag polypeptide, a promoter/regulatory region operably linked to the nucleic acid encoding PCAM-1 not normally present in the cell or not typically operably linked to PCAM-1.

The generation of the non-human transgenic mammal of the invention is preferably accomplished using the method which is now described. However, the invention should in no way be construed as being limited solely to the use of this method, in that, other methods can be used to generate the desired knock-out mammal.

In the preferred method of generating a non-human transgenic mammal, ES cells are generated comprising the transgene of the invention and the cells are then used to generate the knock-out animal essentially as described in

Nagy and Rossant (1993, In: Gene Targeting, A Practical Approach, pp.146-179, Joyner ed., IRL Press). ES cells behave as normal embryonic cells if they are returned to the embryonic environment by injection into a host blastocyst or aggregate with blastomere stage embryos. When so returned, the cells have the full potential to develop along all lineages of the embryo. Thus, it is possible, to obtain ES cells, introduce a desired DNA therein, and then return the cell to the embryonic environment for development into mature mammalian cells, wherein the desired DNA may be expressed.

Precise protocols for the generation of transgenic mice are disclosed in Nagy and Rossant (1993, In: Gene Targeting, A Practical Approach, Joyner ed. IRL Press, pp. 146-179). and are therefore not repeated herein. Transfection or transduction of ES cells in order to introduce the desired DNA therein is accomplished using standard protocols, such as those described, for example, in Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York). Preferably, the desired DNA contained within the transgene of the invention is electroporated into ES cells, and the cells are propagated as described in Soriano et al. (1991, Cell 64:693-702).

Introduction of an isolated nucleic acid into the fertilized egg of the mammal is accomplished by any number of standard techniques in transgenic technology (Hogan et al., 1986, Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor, NY). Most commonly, the nucleic acid is introduced into the embryo by way of microinjection.

Once the nucleic acid is introduced into the egg, the egg is incubated for a short period of time and is then transferred into a pseudopregnant mammal of the same species from which the egg was obtained as described, for example, in Hogan et al. (1986, Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor, NY). Typically, many eggs are injected per experiment, and approximately two-thirds of the eggs survive the procedure. About twenty viable eggs are then transferred into pseudopregnant animals, and usually four to ten of the viable eggs so transferred will develop into live pups.

Any mammalian PCAM-1 gene may be used in the methods described herein to produce a transgenic mammal or a transgenic cell harboring a

transgene comprising a deletion of all or part of that mammalian PCAM-1 gene. Preferably, a rodent PCAM-1 is used.

The transgenic mammal of the invention can be any species of mammal. Thus, the invention should be construed to include generation of transgenic mammals encoding the chimeric nucleic acid, which mammals include mice, hamsters, rats, rabbits, pigs, sheep and cattle. The methods described herein for generation of transgenic mice can be analogously applied using any mammalian species. Preferably, the transgenic mammal of the invention is a rodent and even more preferably, the transgenic mammal of the invention is a mouse. By way of example, Lukkarinen et al. (1997, Stroke 28:639-645), teaches that gene constructs which enable the generation of transgenic mice also enable the generation of other transgenic rodents, including rats. Similarly, nullizygous mutations in a genetic locus of an animal of one species can be replicated in an animal of another species having a genetic locus highly homologous to the first species.

To identify the transgenic mammals of the invention, pups are examined for the presence of the isolated nucleic acid using standard technology such as Southern blot hybridization, PCR, and/or RT-PCR. Expression of the nucleic acid in the cells and in the tissues of the mammal is also assessed using ordinary technology described herein. Further, the presence or absence of PCAM-1 in the circulating blood of the transgenic animal can be determined , for example, as disclosed herein (*e.g.*, Western blot analysis), or using standard methods for protein detection that are well-known in the art.

Cells obtained from the transgenic mammal of the invention, which are also considered "transgenic cells" as the term is used herein, encompass such as cells as those obtained from the PCAM-1 (+/-) and (-/-) transgenic non-human mammal described elsewhere herein, are useful systems for modeling diseases and symptoms of mammals which are believed to be associated with altered levels of PCAM-1 expression such as prostate cancer, and any other disease, disorder or condition associated with an altered level of PCAM-1 expression.

Moreover, as a marker of a pathway(s) associated with tumor proliferation and other abnormalities such prostate, PCAM-1 expression levels are also useful indicators in assessment of such diseases, disorders or conditions.

Particularly suitable are cells derived from a tissue of the non-human knock-out or knock-in transgenic mammal described herein, wherein the transgene comprising the PCAM-1 gene is expressed or inhibits expression of PCAM-1 in various tissues. By way of example, cell types from which such cells are derived include fibroblasts, endothelial, adipocyte, and myoblast cells of (1) the PCAM-1 (+/+), (+/-) and (-/-) non-human transgenic liveborn mammal, (2) the PCAM-1 (+/+), (-/-) or (+/-) fetal animal, and (3) placental cell lines obtained from the PCAM-1 (+/+), (-/-) and (+/-) fetus and liveborn mammal.

One skilled in the art would appreciate, based upon this disclosure, that cells comprising decreased levels of PCAM-1 protein, decreased level of PCAM-1 activity, or both, include, but are not limited to, cells expressing inhibitors of PCAM-1 expression (*e.g.*, antisense or ribozyme molecules).

Methods and compositions useful for maintaining mammalian cells in culture are well known in the art, wherein the mammalian cells are obtained from a mammal including, but not limited to, cells obtained from a mouse such as the transgenic mouse described herein, or cells obtained from primate and non-primate mammals.

The recombinant cell of the invention can be used to produce PCAM-1 for use for therapeutic and/or diagnostic purposes. That is, a recombinant cell expressing PCAM-1 can be used to produce large amounts of purified and isolated PCAM-1 that can be administered to treat or alleviate a disease, disorder or condition associated with or caused by a decreased level of PCAM-1.

Alternatively, recombinant cells expressing PCAM-1 can be administered in *ex vivo* and *in vivo* therapies where administering the recombinant cells thereby administers the protein to a cell, a tissue, and/or an animal. Additionally, the recombinant cells are useful for the discovery of PCAM-1 receptor and PCAM-1 signaling pathways.

The recombinant cell of the invention may be used to study the effects of elevated or decreased PCAM-1 levels on cell homeostasis and cell proliferation since PCAM-1 has been hypothesized to play a role in prostate cancer, and the like

The recombinant cell of the invention, wherein the cell has been engineered such that it does not express PCAM-1, or expresses reduced or altered

PCAM-1 lacking biological activity, can also be used in *ex vivo* and *in vivo* cell therapies where either an animal's own cells (*e.g.*, epithelial cells, fibroblast cells, smooth muscle cells, white blood cells, dendritic cells, and the like) or those of a syngeneic matched donor are recombinantly engineered as described elsewhere herein (*e.g.*, by insertion of an antisense nucleic acid or a knock-out vector such that PCAM-1 expression and/or protein levels are thereby reduced in the recombinant cell), and the recombinant cell is administered to the recipient animal. In this way, recombinant cells that express PCAM-1 at a reduced level can be administered to an animal whose own cells express increased levels of PCAM-1 thereby treating or alleviating a disease, disorder or condition associated with or mediated by increased PCAM-1 expression as disclosed elsewhere herein.

The transgenic mammal of the invention, rendered susceptible to prostate cancer, can be used to study the pathogenesis of prostate cancer and the possible role of PCAM-1 therein.

Further, the transgenic mammal and/or cell of the invention may be used to study the subcellular localization of PCAM-1.

Also, the transgenic mammal (both +/- and -/- live born and fetuses) and/or cell of the invention may be used to study to role(s) of PCAM-1 in glucose metabolism and to elucidate the target(s) of PCAM-1 action as well as any receptor(s) that bind with PCAM-1 to mediate its effect(s) in the cell.

VI. Antibodies

The invention also includes an antibody that specifically binds PCAM-1, or a fragment thereof.

In one embodiment, the antibody is directed to human PCAM-1 comprising the amino acid sequence of SEQ ID NO:2, or an immunogenic portion thereof.

Polyclonal antibodies are generated by immunizing rabbits according to standard immunological techniques well-known in the art (*see, e.g.*, Harlow et al., 1988, In: Antibodies, A Laboratory Manual, Cold Spring Harbor, NY). Such techniques include immunizing an animal with a chimeric protein comprising a portion of another protein such as a maltose binding protein or glutathione (GSH) tag polypeptide portion, and/or a moiety such that the PCAM-1

portion is rendered immunogenic (*e.g.*, PCAM-1 conjugated with keyhole limpet hemocyanin, KLH) and a portion comprising the respective rodent and/or human PCAM-1 amino acid residues. The chimeric proteins are produced by cloning the appropriate nucleic acids encoding PCAM-1 (*e.g.*, SEQ ID NO:1) into a plasmid vector suitable for this purpose, such as but not limited to, pMAL-2 or pCMX.

However, the invention should not be construed as being limited solely to these antibodies or to these portions of the protein antigens. Rather, the invention should be construed to include other antibodies, as that term is defined elsewhere herein, to mouse and human PCAM-1, or portions thereof. Further, the present invention should be construed to encompass antibodies, *inter alia*, bind to PCAM-1 and they are able to bind PCAM-1 present on Western blots, in solution in enzyme linked immunoassays, in fluorescence activated cells sorting (FACS) assays, in immunohistochemical staining of tissues thereby localizing PCAM-1 in the tissues, and in immunofluorescence microscopy of a cell transiently transfected with a nucleic acid encoding at least a portion of PCAM-1.

One skilled in the art would appreciate, based upon the disclosure provided herein, that the antibody can specifically bind with any portion of the protein and the full-length protein can be used to generate antibodies specific therefor. However, the present invention is not limited to using the full-length protein as an immunogen. Rather, the present invention includes using an immunogenic portion of the protein to produce an antibody that specifically binds with mammalian PCAM-1. That is, the invention includes immunizing an animal using an immunogenic portion, or antigenic determinant, of the PCAM-1 protein.

The antibodies can be produced by immunizing an animal such as, but not limited to, a rabbit or a mouse, with a protein of the invention, or a portion thereof, or by immunizing an animal using a protein comprising at least a portion of PCAM-1, or a fusion protein including a tag polypeptide portion comprising, for example, a maltose binding protein tag polypeptide portion, covalently linked with a portion comprising the appropriate PCAM-1 amino acid residues. One skilled in the art would appreciate, based upon the disclosure provided herein, that smaller fragments of these proteins can also be used to produce antibodies that specifically bind PCAM-1.

One skilled in the art would appreciate, based upon the disclosure

provided herein, that various portions of an isolated PCAM-1 polypeptide can be used to generate antibodies to either highly conserved regions of PCAM-1 or to non-conserved regions of the polypeptide including regions containing mutations.

Once armed with the sequence of PCAM-1 and the detailed analysis localizing the various conserved and non-conserved domains of the protein, the skilled artisan would understand, based upon the disclosure provided herein, how to obtain antibodies specific for the various portions of a mammalian PCAM-1 polypeptide using methods well-known in the art or to be developed.

Further, the skilled artisan, based upon the disclosure provided herein, would appreciate that the non-conserved regions of a protein of interest can be more immunogenic than the highly conserved regions which are conserved among various organisms. Further, immunization using a non-conserved immunogenic portion can produce antibodies specific for the non-conserved region thereby producing antibodies that do not cross-react with other proteins which can share one or more conserved portions. Thus, one skilled in the art would appreciate, based upon the disclosure provided herein, that the non-conserved regions of each PCAM-1 molecule can be used to produce antibodies that are specific only for that PCAM-1 and do not cross-react non-specifically with other PCAM-1s or with other proteins.

Alternatively, the skilled artisan would also understand, based upon the disclosure provided herein, that antibodies developed using a region that is conserved among one or more PCAM-1 molecule can be used to produce antibodies that react specifically with one or more PCAM-1 molecule. Methods for producing antibodies that specifically bind with a conserved protein domain which may otherwise be less immunogenic than other portions of the protein are well-known in the art and include, but are not limited to, conjugating the protein fragment of interest to a molecule (*e.g.*, keyhole limpet hemocyanin, and the like), thereby rendering the protein domain immunogenic, or by the use of adjuvants (*e.g.*, Freund's complete and/or incomplete adjuvant, and the like), or both. Thus, the invention encompasses antibodies that recognize at least one PCAM-1 and antibodies that specifically bind with more than one PCAM-1, including antibodies that specifically bind with all PCAM-1s.

One skilled in the art would appreciate, based upon the disclosure

provided herein, which portions of PCAM-1 are less homologous with other proteins sharing conserved domains. However, the present invention is not limited to any particular domain; instead, the skilled artisan would understand that other non-conserved regions of the PCAM-1 proteins of the invention can be used to produce the antibodies of the invention as disclosed herein.

Therefore, the skilled artisan would appreciate, based upon the disclosure provided herein, that the present invention encompasses antibodies that neutralize and/or inhibit PCAM-1 activity (*e.g.*, by necessary PCAM-1/DNA binding interactions, and the like), which antibodies can recognize one or more PCAM-1s.

The invention should not be construed as being limited solely to the antibodies disclosed herein or to any particular immunogenic portion of the proteins of the invention. Rather, the invention should be construed to include other antibodies, as that term is defined elsewhere herein, to PCAM-1, or portions thereof, or to proteins sharing at least about 97% homology with a polypeptide having the amino acid sequence of SEQ ID NO:2. Preferably, the polypeptide is about 98% homologous, and most preferably, about 99% homologous to human PCAM-1 (SEQ ID NO:2). More preferably, the polypeptide that specifically binds with an antibody specific for mammalian PCAM-1 is human PCAM-1. Most preferably, the polypeptide that specifically binds with an antibody that specifically binds with a mammalian PCAM-1 is SEQ ID NO: 2.

The invention encompasses polyclonal, monoclonal, synthetic antibodies, and the like. One skilled in the art would understand, based upon the disclosure provided herein, that the crucial feature of the antibody of the invention is that the antibody bind specifically with PCAM-1. That is, the antibody of the invention recognizes PCAM-1, or a fragment thereof (*e.g.*, an immunogenic portion or antigenic determinant thereof), on Western blots, in immunostaining of cells, and immunoprecipitates PCAM-1 using standard methods well-known in the art.

One skilled in the art would appreciate, based upon the disclosure provided herein, that the antibodies can be used to localize the relevant protein in a cell and to study the role(s) of the antigen recognized thereby in cell processes. Moreover, the antibodies can be used to detect and or measure the amount of protein present in a biological sample using well-known methods such as, but not

limited to, Western blotting and enzyme-linked immunosorbent assay (ELISA). Moreover, the antibodies can be used to immunoprecipitate and/or immuno-affinity purify their cognate antigen using methods well-known in the art. In addition, the antibody can be used to decrease the level of PCAM-1 in a cell thereby inhibiting the effect(s) of PCAM-1 in a cell. Thus, by administering the antibody to a cell or to the tissues of an animal or to the animal itself, the required PCAM-1 receptor/ligand interactions are therefore inhibited such that the effect of PCAM-1 mediated signaling are also inhibited. One skilled in the art would understand, based upon the disclosure provided herein, that detectable effects upon inhibiting PCAM-1 protein/nucleic acid binding interaction using an anti-PCAM-1 antibody can include, but are not limited to, decreased proliferation of prostate tumor cells, and the like.

The skilled artisan would appreciate, based upon the disclosure provided herein, that that present invention includes use of either a single antibody recognizing a single PCAM-1 epitope but that the invention is not limited to use of a single antibody. Instead, the invention encompasses use of at least one antibody where the antibodies can be directed to the same or different PCAM-1 epitopes.

The generation of polyclonal antibodies is accomplished by inoculating the desired animal with the antigen and isolating antibodies which specifically bind the antigen therefrom using standard antibody production methods such as those described in, for example, Harlow et al. (1988, In: Antibodies, A Laboratory Manual, Cold Spring Harbor, NY).

Monoclonal antibodies directed against full length or peptide fragments of a protein or peptide may be prepared using any well known monoclonal antibody preparation procedures, such as those described, for example, in Harlow et al. (1988, In: Antibodies, A Laboratory Manual, Cold Spring Harbor, NY) and in Tuszynski et al. (1988, Blood, 72:109-115). Quantities of the desired peptide may also be synthesized using chemical synthesis technology. Alternatively, DNA encoding the desired peptide may be cloned and expressed from an appropriate promoter sequence in cells suitable for the generation of large quantities of peptide. Monoclonal antibodies directed against the peptide are generated from mice immunized with the peptide using standard procedures as referenced herein.

Nucleic acid encoding the monoclonal antibody obtained using the procedures described herein may be cloned and sequenced using technology which is available in the art, and is described, for example, in Wright et al. (1992, Critical Rev. Immunol. 12:125-168), and the references cited therein.

Further, the antibody of the invention may be "humanized" using the technology described in, for example, Wright et al., *id.*, and in the references cited therein, and in Gu et al. (1997, Thrombosis and Hematocyst 77:755-759), and other methods of humanizing antibodies well-known in the art or to be developed.

To generate a phage antibody library, a cDNA library is first obtained from mRNA which is isolated from cells, *e.g.*, the hybridoma, which express the desired protein to be expressed on the phage surface, *e.g.*, the desired antibody. cDNA copies of the mRNA are produced using reverse transcriptase. cDNA which specifies immunoglobulin fragments are obtained by PCR and the resulting DNA is cloned into a suitable bacteriophage vector to generate a bacteriophage DNA library comprising DNA specifying immunoglobulin genes. The procedures for making a bacteriophage library comprising heterologous DNA are well known in the art and are described, for example, in Sambrook et al., *supra*.

Bacteriophage which encode the desired antibody, may be engineered such that the protein is displayed on the surface thereof in such a manner that it is available for binding to its corresponding binding protein, *e.g.*, the antigen against which the antibody is directed. Thus, when bacteriophage which express a specific antibody are incubated in the presence of a cell which expresses the corresponding antigen, the bacteriophage will bind to the cell. Bacteriophage which do not express the antibody will not bind to the cell. Such panning techniques are well known in the art and are described for example, in Wright et al. (*supra*).

Processes such as those described above, have been developed for the production of human antibodies using M13 bacteriophage display (Burton et al., 1994, Adv. Immunol. 57:191-280). Essentially, a cDNA library is generated from mRNA obtained from a population of antibody-producing cells. The mRNA encodes rearranged immunoglobulin genes and thus, the cDNA encodes the same. Amplified cDNA is cloned into M13 expression vectors creating a library of phage which express human Fab fragments on their surface. Phage which display the

antibody of interest are selected by antigen binding and are propagated in bacteria to produce soluble human Fab immunoglobulin. Thus, in contrast to conventional monoclonal antibody synthesis, this procedure immortalizes DNA encoding human immunoglobulin rather than cells which express human immunoglobulin.

The procedures just presented describe the generation of phage which encode the Fab portion of an antibody molecule. However, the invention should not be construed to be limited solely to the generation of phage encoding Fab antibodies. Rather, phage which encode single chain antibodies (scFv/phage antibody libraries) are also included in the invention. Fab molecules comprise the entire Ig light chain, that is, they comprise both the variable and constant region of the light chain, but include only the variable region and first constant region domain (CH1) of the heavy chain. Single chain antibody molecules comprise a single chain of protein comprising the Ig Fv fragment. An Ig Fv fragment includes only the variable regions of the heavy and light chains of the antibody, having no constant region contained therein. Phage libraries comprising scFv DNA may be generated following the procedures described in Marks et al. (1991, J. Mol. Biol. 222:581-597). Panning of phage so generated for the isolation of a desired antibody is conducted in a manner similar to that described for phage libraries comprising Fab DNA.

The invention should also be construed to include synthetic phage display libraries in which the heavy and light chain variable regions may be synthesized such that they include nearly all possible specificities (Barbas, 1995, Nature Medicine 1:837-839; de Kruif et al. 1995, J. Mol. Biol. 248:97-105).

One skilled in the art would appreciate, based upon the disclosure provided herein, that present invention encompasses an immunotoxin comprising an antibody component that specifically binds with PCAM-1 linked to another agent, particularly a cytotoxic or otherwise anticellular agent, having the ability to kill or suppress the growth or cell division of cells. Such immunotoxins, or immuno-conjugates, are well known in the art and there are a plethora of toxic agents that can be used to produce them such as, but not limited to, ricin toxin, *staphylococcal* enterotoxin A (SEA) (Dohlsten et al., 1994, Proc. Natl. Acad. Sci. USA 91:8945-8949), the plant toxin gelonin (Rosenblum et al., U.S. Pat. No. 5,624,827), *Pseudomonas* exotoxin (PE), and the like. Therefore, the invention encompasses

use of antibodies that specifically bind with PCAM-1 to preferentially target cytotoxic agents to tumor cells while minimizing the cytotoxic effect(s) to normal cells and tissues since, as disclosed herein, tumor cells express higher level of PCAM-1 than normal, non-tumor cells.

VII. Compositions

The invention includes a composition comprising an isolated nucleic complementary to a nucleic acid, or a portion thereof, encoding a mammalian PCAM-1 which is in an antisense orientation with respect to transcription. Preferably, the composition comprises a pharmaceutically acceptable carrier.

The invention includes a composition comprising an isolated nucleic complementary to a nucleic acid, or a portion thereof, encoding a mammalian PCAM-1 which is a ribozyme that specifically cleaves PCAM-1. Preferably, the composition comprises a pharmaceutically acceptable carrier.

The invention includes a composition comprising an isolated mammalian PCAM-1 polypeptide as described herein. Preferably, the composition comprises a pharmaceutically-acceptable carrier.

The invention also includes a composition comprising an antibody that specifically binds PCAM-1. Preferably, the composition comprises a pharmaceutically-acceptable carrier.

The invention further includes a composition comprising an isolated nucleic acid encoding a mammalian PCAM-1. Preferably, the composition comprises a pharmaceutically acceptable carrier. The compositions can be used to administer PCAM-1, and/or a nucleic acid encoding the protein, to a cell, a tissue, or an animal or to inhibit expression of PCAM-1 in a cell, a tissue, or an animal. The compositions are useful to treat a disease, disorder or condition mediated by altered expression of PCAM-1 such that decreasing or increasing PCAM-1 expression or the level of the protein in a cell, tissue, or animal, is beneficial to the animal. That is, where a disease, disorder or condition in an animal is mediated by or associated with altered level of PCAM-1 expression or protein level, the composition can be used to modulate such expression or protein level of PCAM-1.

One skilled in the art would understand, based on the disclosure provided herein, that PCAM-1 can be administered to a cell or tissue by

administering the protein itself or by administering a nucleic acid encoding the protein. Either way, PCAM-1 is administered to the cell and/or tissue.

For administration to the mammal, a polypeptide, or a nucleic acid encoding it, a ribozyme that specifically cleaves an mRNA encoding the polypeptide, and/or an antisense nucleic acid complementary to all or a portion of a nucleic acid encoding the protein, can be suspended in any pharmaceutically acceptable carrier, for example, HEPES buffered saline at a pH of about 7.8.

The skilled artisan would further appreciate, based upon the disclosure provided herein, that the invention encompasses compositions comprising at least one of a nucleic acid encoding PCAM-1, an isolated PCAM-1 polypeptide, an antisense nucleic acid complementary with a nucleic acid encoding PCAM-1, an enzymatic nucleic acid that specifically cleaves RNA transcribed from a nucleic acid encoding PCAM-1, and an antibody that specifically binds with PCAM-1, or a portion thereof.

The compositions encompassed in the invention also comprise those comprising various antibodies that specifically bind with various epitopes of the PCAM-1 polypeptide, ribozymes that specifically bind with and cleave various portions of the RNA encoding PCMA-1, double-stranded oligonucleotides that specifically bind with separate and/or overlapping regions of a nucleic acid encoding PCAM-1, and antisense nucleic acid molecules complementary to various different portions of a nucleic acid encoding PCAM-1.

One skilled in the art would understand, based upon the instant disclosure, that compositions comprising mixtures of the above-discussed compounds, *i.e.*, ribozymes, antisense nucleic acids, antibodies, nucleic acids encoding PCAM-1, and PCAM-1 polypeptides, double-stranded oligonucleotides that specifically bind with PCAM-1 polypeptide, and the like, are encompassed in the invention.

Additionally, compositions comprising at least one of the afore-mentioned compounds where the compositions further comprise additional compounds, such as, but not limited to, small molecules, peptidomimetics, ribozymes and antisense nucleic acids specific for other proteins (*e.g.*, VEGF-1 and MMP-2, and the like), drugs, chemotherapeutic agents, and the like, are also contemplated in the present invention. One skilled in the art would appreciate,

based upon the disclosure provided herein, that such compositions are useful for diagnosis and treatment of diseases, disorders, or conditions associated with or mediated by altered expression of PCAM-1.

Other pharmaceutically acceptable carriers which are useful include, but are not limited to, glycerol, water, saline, ethanol and other pharmaceutically acceptable salt solutions such as phosphates and salts of organic acids. Examples of these and other pharmaceutically acceptable carriers are described in Remington's Pharmaceutical Sciences (1991, Mack Publication Co., New Jersey).

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides.

Pharmaceutical compositions that are useful in the methods of the invention may be administered, prepared, packaged, and/or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

The compositions of the invention may be administered via numerous routes, including, but not limited to, oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, or ophthalmic administration routes. The route(s) of administration will be readily apparent to the skilled artisan and will depend upon any number of factors including the type and severity of the disease being treated, the type and age of the veterinary or human patient being treated, and the like.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in oral solid formulations, ophthalmic,

suppository, aerosol, topical or other similar formulations. In addition to the compound such as heparan sulfate, or a biological equivalent thereof, such pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer PCAM-1 and/or a nucleic acid encoding the same according to the methods of the invention.

Compounds which are identified using any of the methods described herein may be formulated and administered to a mammal for treatment of prostate cancer are now described.

The invention encompasses the preparation and use of pharmaceutical compositions comprising a compound useful for treatment of prostate cancer as an active ingredient. Such a pharmaceutical composition may consist of the active ingredient alone, in a form suitable for administration to a subject, or the pharmaceutical composition may comprise the active ingredient and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The active ingredient may be present in the pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

As used herein, the term "pharmaceutically acceptable carrier" means a chemical composition with which the active ingredient may be combined and which, following the combination, can be used to administer the active ingredient to a subject.

As used herein, the term "physiologically acceptable" ester or salt means an ester or salt form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition, which is not deleterious to the subject to which the composition is to be administered.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory

ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs.

Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, intrathecal or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents. Particularly contemplated additional agents include anti-emetics and scavengers such as cyanide and cyanate scavengers.

Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

A formulation of a pharmaceutical composition of the invention suitable for oral administration may be prepared, packaged, or sold in the form of a discrete solid dose unit including, but not limited to, a tablet, a hard or soft capsule, a cachet, a troche, or a lozenge, each containing a predetermined amount of the active ingredient. Other formulations suitable for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, or an emulsion.

As used herein, an "oily" liquid is one which comprises a carbon-containing liquid molecule and which exhibits a less polar character than water.

A tablet comprising the active ingredient may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing, in a suitable device, the active ingredient in a free-flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture. Pharmaceutically acceptable excipients used in the manufacture of tablets include, but are not limited to, inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycollate. Known surface active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include, but are not limited to, corn starch and alginic acid. Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone,

and hydroxypropyl methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

Tablets may be non-coated or they may be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the active ingredient. By way of example, a material such as glyceryl monostearate or glyceryl distearate may be used to coat tablets. Further by way of example, tablets may be coated using methods described in U.S. Patents numbers 4,256,108; 4,160,452; and 4,265,874 to form osmotically-controlled release tablets. Tablets may further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide pharmaceutically elegant and palatable preparation.

Hard capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the active ingredient, and may further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the active ingredient, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

Liquid formulations of a pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings,

coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (*e.g.*, polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-para- hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative.

Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for rectal administration. Such a composition may be in the form of, for example, a suppository, a retention enema preparation, and a solution for rectal or colonic irrigation.

Suppository formulations may be made by combining the active ingredient with a non-irritating pharmaceutically acceptable excipient which is solid at ordinary room temperature (*i.e.*, about 20°C) and which is liquid at the rectal temperature of the subject (*i.e.*, about 37°C in a healthy human). Suitable pharmaceutically acceptable excipients include, but are not limited to, cocoa butter, polyethylene glycols, and various glycerides. Suppository formulations may further comprise various additional ingredients including, but not limited to, antioxidants and preservatives.

Retention enema preparations or solutions for rectal or colonic irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, enema preparations may be administered using, and may be packaged within, a delivery device adapted to the rectal anatomy of the subject. Enema preparations may further comprise various additional ingredients including, but not limited to, antioxidants and preservatives.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for vaginal administration. Such a

composition may be in the form of, for example, a suppository, an impregnated or coated vaginally-insertable material such as a tampon, a douche preparation, or gel or cream or a solution for vaginal irrigation.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (*i.e.*, such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

Douche preparations or solutions for vaginal irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, douche preparations may be administered using, and may be packaged within, a delivery device adapted to the vaginal anatomy of the subject. Douche preparations may further comprise various additional ingredients including, but not limited to, antioxidants, antibiotics, antifungal agents, and preservatives.

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers

containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (*i.e.*, powder or granular) form for reconstitution with a suitable vehicle (*e.g.*, sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 to about 7 nanometers, and preferably from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder or using a self-propelling solvent/powder-dispensing container such as a device comprising the active ingredient dissolved or suspended in a low-boiling propellant in a sealed container. Preferably, such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter less than 7 nanometers. More preferably, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. Dry powder compositions preferably include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

Low boiling propellants generally include liquid propellants having a boiling point of below 65°F at atmospheric pressure. Generally the propellant may constitute 50 to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1 to 20% (w/w) of the composition. The propellant may further comprise additional ingredients such as a liquid non-ionic or solid anionic surfactant or a solid diluent (preferably having a particle size of the same order as particles comprising the active ingredient).

Pharmaceutical compositions of the invention formulated for pulmonary delivery may also provide the active ingredient in the form of droplets of a solution or suspension. Such formulations may be prepared, packaged, or sold as aqueous or dilute alcoholic solutions or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, or a preservative such as methylhydroxybenzoate. The droplets provided by this route

of administration preferably have an average diameter in the range from about 0.1 to about 200 nanometers.

The formulations described herein as being useful for pulmonary delivery are also useful for intranasal delivery of a pharmaceutical composition of the invention.

Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 to 500 micrometers. Such a formulation is administered in the manner in which snuff is taken, *i.e.*, by rapid inhalation through the nasal passage from a container of the powder held close to the nares.

Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may, for example, 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1-1.0% (w/w) solution or suspension of the active ingredient in an aqueous or oily liquid carrier. Such drops may further comprise buffering agents, salts, or one or more other of the additional ingredients described herein. Other ophthalmalmically-administrable formulations which are useful include those

which comprise the active ingredient in microcrystalline form or in a liposomal preparation.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed. (1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA), which is incorporated herein by reference.

Typically, dosages of the compound of the invention which may be administered to an animal, preferably a human, will vary depending upon any number of factors, including but not limited to, the type of animal and type of disease state being treated, the age of the animal and the route of administration.

The compound can be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even lees frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

VIII. Methods

A. Methods of identifying useful compounds

The present invention further includes a method of identifying a compound that affects expression of PCAM-1 in a cell. The method comprises contacting a cell with a test compound and comparing the level of expression of PCAM-1 in the cell so contacted with the level of expression of PCAM-1 in an

otherwise identical cell not contacted with the compound. If the level of expression of PCAM-1 is higher or lower in the cell contacted with the test compound compared to the level of expression of PCAM-1 in the otherwise identical cell not contacted with the test compound, this is an indication that the test compound affects expression of PCAM-1 in a cell.

Similarly, the present invention includes a method of identifying a compound that reduces expression of PCAM-1 in a cell. The method comprises contacting a cell with a test compound and comparing the level of expression of PCAM-1 in the cell contacted with the compound with the level of expression of PCAM-1 in an otherwise identical cell, which is not contacted with the compound. If the level of expression of PCAM-1 is lower in the cell contacted with the compound compared to the level in the cell that was not contacted with the compound, then that is an indication that the test compound affects reduces expression of PCAM-1 in a cell.

One skilled in the art would appreciate, based on the disclosure provided herein, that the level of expression of PCAM-1 in the cell may be measured by determining the level of expression of mRNA encoding PCAM-1. Alternatively, the level of expression of mRNA encoding PCAM-1 can be determined by using immunological methods to assess PCAM-1 production from such mRNA as exemplified herein using Western blot analysis, FACS analysis, or enzyme linked immunoassays using an anti-PCAM-1 antibody of the invention. Further, nucleic acid-based detection methods, such as Northern blot and PCR assays and the like, can be used as well. Thus, one skilled in the art would appreciate, based upon the extensive disclosure and reduction to practice provided herein, that there are a plethora of methods that are well-known in the art, which can be used to asses the level of expression of PCAM-1 in a cell including those disclosed herein and others which may be developed in the future.

Further, one skilled in the art would appreciate based on the disclosure provided herein that, as disclosed in the examples below, a cell which lacks endogenous PCAM-1 expression can be transfected with a vector comprising an isolated nucleic acid encoding PCAM-1 whereby expression of PCAM-1 is effected in the cell. The transfected cell is then contacted with the test compound thereby allowing the determination of whether the compound affects the expression

of PCAM-1. Therefore, one skilled in the art armed with the present invention would be able to, by selectively transfecting a cell lacking detectable levels of PCAM-1 using PCAM-1-expressing vectors, identify a compound which selectively affects PCAM-1 expression.

One skilled in the art would understand, based upon the disclosure provided herein, that the invention encompasses any test compound identified using the methods discussed elsewhere herein. That is, a compound that inhibits PCAM-1 expression can be used to develop therapeutics and diagnostics for diseases, disorders or conditions mediated by PCAM-1 over-expression such as prostate cancer. That is, one skilled in the art would appreciate, as more fully set forth elsewhere herein in discussing ribozymes that specifically cleave PCAM-1, that decreasing the level of PCAM-1 expression associated with a disease, disorder or condition is a potential therapeutic for treatment of the disease, disorder or condition. Thus, a compound identified by the methods disclosed herein is a potential therapeutic for treatment of prostate cancer, among other things.

One skilled in the art would understand, based upon the disclosure provided herein, that the invention encompasses methods of identifying a compound that increases the level of PCAM-1 in a cell. These methods are useful in that the data disclosed herein demonstrate, for the first time, that increased expression of PCAM-1 is associated with and/or mediates prostate cancer. Thus, a compound that increases the level of PCAM-1 is a potential prostate carcinogen and the identification of such compounds is important in assessing the potential toxicity of a compound and is thus a useful assay, for example, in the field of drug development where the identification of potential deleterious effects associated with a novel compound is of utmost importance. Therefore, the present invention provides useful assays for identification of potential negative effect in the field of drug development, and the like.

The skilled artisan would further appreciate, based upon the disclosure provided herein, that the present invention includes a method of identifying a compound that inhibits binding of PCAM-1 with a double-stranded nucleic acid that specifically binds with PCAM-1. The method comprises assessing the level of PCAM-1 binding with a double-stranded nucleic acid known to specifically bind with PCAM-1. Such double-stranded nucleic acids include, but

are not limited to, a nucleic acid having the sequence SEQ ID NO:5, sequence SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8. That is, by assessing and comparing the level of PCAM-1 binding with a double-stranded nucleic acid that specifically binds with PCAM-1 in the presence and absence of a compound, a compound can be identified where the level of binding of PCAM-1 with the nucleic acid is lower in the presence of the compound compared with the level in the absence of the compound. Thus, a compound that inhibits PCAM-1 binding with a nucleic acid that specifically binds with PCAM-1 can be identified and such assays are encompassed in the present invention. These compounds may be useful therapeutics since the specific binding interaction between PCAM-1 and a nucleic acid that specifically binds therewith can be a potential target for treatment of a disease, disorder or disease associated with or mediated by such binding interaction, *e.g.*, prostate cancer, and the like.

B. Methods of treating or alleviating a disease, disorder or condition associated with or mediated by PCAM-1 expression

The invention includes a method of alleviating a disease, disorder or condition mediated by mal-expression of PCAM-1. The method comprises administering an expression modulating compound, *e.g.*, an antisense nucleic acid or ribozyme complementary to a nucleic acid encoding PCAM-1, to a patient afflicted with a disease, disorder or condition mediated by increased PCAM-1 expression compared to the level of PCAM-1 expression in otherwise identical but normal tissue, *i.e.*, tissue which does not exhibit any detectable clinical parameters associated with the disease, disorder or condition being treated or alleviated. This, in turn, mediates a decrease in PCAM-1 expression thereby alleviating a disease, disorder or condition mediated by mal-expression of PCAM-1. Such diseases, disorder or conditions include, but are not limited to, prostate cancer.

Antisense nucleic acids and ribozymes that inhibit expression of PCAM-1 can therefore also be used for the manufacture of a medicament for treatment of a disease, disorder or condition mediated by increased expression of PCAM-1 when compared with expression of PCAM-1 in a cell and/or a patient not afflicted with the disease, disorder or condition.

One skilled in the art would understand, based upon the disclosure provided herein, that because reducing expression of PCAM-1 can mediate a beneficial effect in a patient afflicted with prostate cancer, decreased PCAM-1 expression can be useful for treating such diseases, disorders, or conditions. This is because, as disclosed elsewhere herein, increased expression of PCAM-1 is associated with abnormal cell proliferation associated with prostate cancer. Further, the data disclosed elsewhere herein demonstrate that inhibition of PCAM-1 expression, such as by administration of a ribozyme that specifically cleaves PCAM-1 mRNA, effected a beneficial decrease in tumors and increased the survival time in an art-recognized mouse model for study of prostate cancer therapeutics. Thus, one skilled in the art would appreciate, based upon the disclosure provided herein, that inhibition of PCAM-1 expression can inhibit the deleterious effects of PCAM-1 mal-expression.

One skilled in the art would understand, based upon the disclosure provided herein, that since reduced PCAM-1 expression can mediate a beneficial effect, methods of decreasing expression of PCAM-1, decreasing the level of PCAM-1 polypeptide present in the cell, and/or decreasing the activity of PCAM-1 in a cell (using, *e.g.*, antisense nucleic acids, ribozymes, antibodies, and the like), can be used to treat and/or alleviate a disease, disorder or condition associated with altered expression of PCAM-1 where a lower level of PCAM-1 would provide a benefit. Thus, whether an antisense nucleic acid, a ribozyme, or a blocking antibody is administered, the crucial feature of the present invention is that the expression of PCAM-1 be reduced in a cell.

Techniques for inhibiting expression of a nucleic acid in a cell are well known in the art and encompass such methods as disclosed herein (*e.g.*, inhibition using an antibody, an antisense nucleic acid, a ribozyme, and the like). Other techniques useful for inhibiting expression of a nucleic acid encoding PCAM-1 include, but are not limited to, using nucleotide reagents that target specific sequences of the PCAM-1 promoter, and the like.

One skilled in the art would understand, based upon the disclosure provided herein, that it may be useful to increase the level or activity of PCAM-1 in a cell. That is, it can be useful to treat or alleviate a disease, disorder of condition associated with or mediated by decreased expression, level, or activity of PCAM-1

by administering PCAM-1. Such diseases, disorders or conditions include, but are not limited to prostate cancer, and possibly other solid cancers or leukemias, AIDS, HIV infection , immune disorders and inflammatory or degenerative disorders, and the like.

Whether expression of PCAM-1, levels of the polypeptide, or its activity, is increased or decreased, one skilled in the art would appreciate, based on this disclosure, that methods of reducing or inducing PCAM-1 of the invention encompass administering a recombinant cell that either expresses or lacks expression of PCAM-1.

In another embodiment of the invention, an individual suffering from a disease, disorder or a condition that is associated with or mediated by altered PCAM-1 expression can be treated by supplementing, augmenting and/or replacing defective cells with cells that lack PCAM-1 expression. The cells can be derived from cells obtained from a normal syngeneic matched donor or cells obtained from the individual to be treated. The cells may be genetically modified to inhibit PCAM-1 expression.

An example of a disease, disorder or a condition associated with or mediated by PCAM-1 expression is prostate cancer, and the like.

In addition to replacing defective cells with repaired cells or normal cells from matched donors, the method of the invention may also be used to facilitate expression of a desired protein that when secreted in the an animal, has a beneficial effect. That is, cells may be isolated, furnished with a gene encoding PCAM-1 and introduced into the donor or into a syngeneic matched recipient. Expression of the PCAM-1 exerts a therapeutic effect.

This aspect of the invention relates to gene therapy in which therapeutic amounts of PCAM-1 are administered to an individual.

According to some aspects of the present invention, recombinant cells transfected with either nucleic acid encoding PCAM-1, antisense nucleic acids, a nucleic acid encoding a ribozyme, or a knock-out targeting vector of the invention, can be used as cell therapeutics to treat a disease, disorder or a condition characterized by expression of PCAM-1, or the lack thereof.

In particular, a gene construct that comprises a heterologous gene which encodes PCAM-1 is introduced into cells. These recombinant cells are used

to purify isolated PCAM-1, which was then administered to an animal. One skilled in the art would understand, based upon the disclosure provided herein, that instead of administering an isolated PCAM-1 polypeptide, PCAM-1 can be administered to a mammal in need thereof by administering to the mammal the recombinant cells themselves. This will benefit the recipient individual who will benefit when the protein is expressed and secreted by the recombinant cell into the recipient's system.

According to the present invention, gene constructs comprising nucleotide sequences of the invention are introduced into cells. That is, the cells, referred to herein as "recombinant cells," are genetically altered to introduce a nucleic acid encoding PCAM-1 or a nucleic acid that inhibits PCAM-1 expression in and/or secretion by the recombinant cell (*e.g.*, an antisense nucleic acid, an enzymatic nucleic acid that specifically cleaves RNA transcribed from a nucleic acid encoding a PCAM-1) thereby mediating a beneficial effect on an recipient to which the recombinant cell is administered. According to some aspects of the invention, cells obtained from the same individual to be treated or from another individual, or from a non-human animal, can be genetically altered to replace a defective gene and/or to introduce a nucleic acid whose expression has a beneficial effect on the individual or to inhibit PCAM-1 expression which inhibition can have a beneficial effect on the individual.

In some aspects of the invention, an individual suffering from a disease, disorder or a condition can be treated by supplementing, augmenting and/or replacing defective or deficient nucleic acid encoding PCAM-1 by providing an isolated recombinant cells containing gene constructs that include normal, functioning copies of a nucleic acid encoding PCAM-1. This aspect of the invention relates to gene therapy in which the individual is provided with a nucleic encoding PCAM-1 for which they are deficient in presence and/or function. The isolated nucleic acid encoding PCAM-1 provided by the cell compensates for the defective PCAM-1 expression of the individual, because, when the nucleic acid is expressed in the individual, a protein is produced which serves to alleviate or otherwise treat the disease, disorder or condition in the individual. Such nucleic acid preferably encodes a PCAM-1 polypeptide that is secreted from the recombinant cell.

In all cases in which a gene construct encoding PCAM-1 is transfected into a cell, the nucleic acid is operably linked to an appropriate promoter/regulatory sequence which is required to achieve expression of the nucleic acid in the recombinant cell. Such promoter/regulatory sequences include but are not limited to, constitutive and inducible and/or tissue specific and differentiation specific promoters, and are discussed elsewhere herein. Constitutive promoters include, but are not limited to, the cytomegalovirus immediate early promoter and the Rous sarcoma virus promoter. In addition, housekeeping promoters such as those which regulate expression of housekeeping genes may also be used. Other promoters include those which are preferentially expressed in cells of the central nervous system, such as, but not limited the promoter for the gene encoding glial fibrillary acidic protein. In addition, promoter/regulatory elements may be selected such that gene expression is inducible. For example, a tetracycline inducible promoter may be used (Freundlich et al., 1997, Meth. Enzymol. 283:159-173).

The gene construct is preferably provided as an expression vector which includes the coding sequence of a mammalian PCAM-1 of the invention operably linked to essential promoter/regulatory sequences such that when the vector is transfected into the cell, the coding sequence is expressed by the cell. The coding sequence is operably linked to the promoter/regulatory elements necessary for expression of the sequence in the cells. The nucleotide sequence that encodes the protein may be cDNA, genomic DNA, synthesized DNA or a hybrid thereof or an RNA molecule such as mRNA.

The gene construct, which includes the nucleotide sequence encoding PCAM-1 operably linked to the promoter/regulatory elements, may remain present in the cell as a functioning episomal molecule or it may integrate into the chromosomal DNA of the cell. Genetic material may be introduced into cells where it remains as separate genetic material in the form of a plasmid. Alternatively, linear DNA which can integrate into a host cell chromosome may be introduced into the cell. When introducing DNA into the cell, reagents which promote DNA integration into chromosomes may be added. DNA sequences which are useful to promote integration may also be included in the DNA molecule. Alternatively, RNA may be introduced into the cell.

In order for genetic material in an expression vector to be expressed,

the promoter/regulatory elements must be operably linked to the nucleotide sequence that encodes the protein. In order to maximize protein production, promoter/regulatory sequences may be selected which are well suited for gene expression in the desired cells. Moreover, codons may be selected which are most efficiently transcribed in the cell. One having ordinary skill in the art can produce recombinant genetic material as expression vectors which are functional in the desired cells.

It is also contemplated that promoter/regulatory elements may be selected to facilitate tissue specific expression of the protein. Thus, for example, specific promoter/regulatory sequences may be provided such that the heterologous gene will only be expressed in the tissue where the recombinant cells are implanted. One skilled in the art would understand, based upon the disclosure provided herein, that the preferred tissues where the expression or lack of expression of PCAM-1 is to be targeted include, but are not limited to, prostate tissue. In addition, promoter/regulatory elements may be selected such that gene expression is inducible. For example, a tetracycline inducible promoter may be used (Freundlich et al., 1997, Meth. Enzymol. 283:159-173).

In addition to providing cells with recombinant genetic material that either corrects a genetic defect in the cells, that encodes a protein which is otherwise not present in sufficient quantities and/or functional condition so that the genetic material corrects a genetic defect in the individual, and/or that encodes a protein which is useful as beneficial in the treatment or prevention of a particular disease, disorder or condition associated therewith, and that inhibits expression of PCAM-1 in the cell (*e.g.*, a knock-out targeting vector, an antisense nucleic acid, a ribozyme, and the like), genetic material can also be introduced into the recombinant cells used in the present invention to provide a means for selectively terminating such cells should such termination become desirable. Such means for targeting recombinant cells for destruction may be introduced into recombinant cells.

According to the invention, recombinant cells can be furnished with genetic material which renders them specifically susceptible to destruction. For example, recombinant cells may be provided with a gene that encodes a receptor that can be specifically targeted with a cytotoxic agent. An expressible form of a

gene that can be used to induce selective cell death can be introduced into the recombinant cells. In such a system, cells expressing the protein encoded by the gene are susceptible to targeted killing under specific conditions or in, the presence or absence of specific agents. For example, an expressible form of a herpes virus thymidine kinase (herpes tk) gene can be introduced into the recombinant cells and used to induce selective cell death. When the introduced genetic material that includes the herpes tk gene is introduced into the individual, herpes tk will be produced. If it is desirable or necessary to kill the implanted recombinant cells, the drug gangcyclovir can be administered to the individual which will cause the selective killing of any cell producing herpes tk. Thus, a system can be provided which allows for the selective destruction of implanted recombinant cells.

One skilled in the art would understand, based upon the disclosure provided herein, that the present invention encompasses production of recombinant cells to either provide PCAM-1 to or inhibit PCAM-1 expression in a mammal. That is, the cells can be used to administer PCAM-1 to an animal or to deliver a molecule (*e.g.*, a knock-out targeting vector, an antisense nucleic acid, a ribozyme [*e.g.*, an isolated enzymatic nucleic acid having the sequence of SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11], and antibody that specifically binds with PCAM-1, and the like).

Administration of PCAM-1 to an animal can be used as a model system to study the mechanism of action of PCAM-1, *e.g.*, for assessing the effect(s) of inhibiting PCAM-1/DNA binding interactions, and to develop model systems useful for the development of diagnostics and/or therapeutics for diseases, disorders or conditions associated with PCAM-1 expression.

Further, the delivery of PCAM-1 to an animal mediated by administration of recombinant cells expressing and secreting PCAM-1 can also be used to treat or alleviate a disease, disorder or condition where increasing the level of PCAM-1 mediates a therapeutic effect. More specifically, administration of PCAM-1 to an animal by administering a recombinant cell expressing a nucleic acid encoding PCAM-1 can be useful for treatment of prostate cancer (*i.e.*, in dogs and humans) prostate cancer, and possibly other solid cancers or leukemias, AIDS, HIV infection, immune disorders, and inflammatory or degenerative disorders which afflict humans and animals alike, among other things.

Alternatively, administration of recombinant cells comprising a nucleic acid the expression of which inhibits or reduces PCAM-1 expression, activity, and/or PCAM-1 binding with DNA, can be used as a model for the development of diagnostics and/or therapeutics useful for diseases, disorders or conditions associated with or mediated by PCAM-1 expression, activity, and/or protein/nucleic acid binding interactions. The present invention encompasses that the recombinant cells can produce the molecule that inhibits PCAM-1 expression thereby providing such molecule to the animal. Alternatively, without wishing to be bound by any particular theory, the recombinant cells themselves, which are otherwise functional cells, except for the inability to express PCAM-1, can perform the functions of otherwise identical but non-recombinant cells, without being subject to the PCAM-1 signaling pathway.

Cells, whether obtained from an animal, from established cell lines that are commercially available or to be developed, or primary cells cultured *in vitro*, can be transfected using well known techniques readily available to those having ordinary skill in the art. Thus, the present invention is not limited to obtaining cells from a donor animal or from the patient animal itself. Rather, the invention includes using any cell that can be engineered using a nucleic acid of the invention such that the recombinant cell either expresses PCAM-1, a PCAM-1 ribozyme, PCAM-1 antisense nucleic acid, and/or antibody that specifically binds with PCAM-1 (where it did not express such molecule prior to being engineered, or where the cell produced the molecule an a different level prior to the introduction of the nucleic acid into the cell) or the recombinant cell does not express PCAM-1, PCAM-1 ribozyme, PCAM-1 antisense, and/or antibody that specifically binds with PCAM-1 or expresses it at a lower level (where it expressed the molecule before or expressed it a different level prior to introduction of the nucleic acid into the cell).

Nucleic acids can be introduced into the cells using standard methods which are employed for introducing a gene construct into cells which express the protein encoded by the gene or which express a molecule that inhibits PCAM-1 expression. In some embodiments, cells are transfected by calcium phosphate precipitation transfection, DEAE dextran transfection, electroporation, microinjection, liposome-mediated transfer, chemical-mediated transfer, ligand mediated transfer or recombinant viral vector transfer.

In some embodiments, recombinant adenovirus vectors are used to introduce DNA having a desired sequence into the cell. In some embodiments, recombinant retrovirus vectors are used to introduce DNA having a desired sequence into the cell. In some embodiments, standard calcium phosphate, DEAE dextran or lipid carrier mediated transfection techniques are employed to incorporate a desired DNA into dividing cells. Standard antibiotic resistance selection techniques can be used to identify and select transfected cells. In some embodiments, DNA is introduced directly into cells by microinjection. Similarly, well known electroporation or particle bombardment techniques can be used to introduce foreign DNA into cells. A second gene is usually co-transfected with and/or covalently linked to the nucleic acid encoding PCAM-1, or knock-out targeting vector, ribozyme, or antisense molecule thereto. The second gene is frequently a selectable antibiotic-resistance gene. Transfected recombinant cells can be selected by growing the cells in an antibiotic that kills cells that do not take up the selectable gene. In most cases where the two genes are unlinked and co-transfected, the cells that survive the antibiotic treatment contain and express both genes.

Where an isolated PCAM-1 polypeptide, an antibody that specifically binds with PCAM-1, a PCAM-1 antisense nucleic acid, a PCAM-1 ribozyme, and/or recombinant cells of the invention are administered to an animal either to increase or reduce the level of PCAM-1 present in the animal, one skilled in the art would understand, based upon the disclosure provided herein, that the amount of the polypeptide, nucleic acid, antibody, or cell to be administered to the animal can be titrated by assessing the level of PCAM-1 and/or sugar present in the blood or by determining the level of expression of PCAM-1 or the level of PCAM-1 polypeptide or nucleic acid encoding PCAM-1 present in the tissues of the animal.

Further, the skilled artisan would understand, based upon the disclosure provided herein, that a mixture of any compound that inhibits the effect of PCAM-1 (*e.g.*, an antisense, a PCAM-1 ribozyme, an antibody, a double-stranded nucleic acid that specifically binds with PCAM-1 thereby disrupting PCAM-1/DNA binding necessary for PCAM-1 activity) can also be used to alleviate and/or treat a disease, disorder or condition associated with or mediated by altered PCAM-1 expression. Further, one or more such compounds can be

combined with other compounds useful to treat diseases, disorders or conditions such as prostate cancer. That is, the invention encompasses administration of PCAM-1 antisense nucleic acid, PCAM-1 ribozyme, anti-PCAM-1 antibody, and double-stranded nucleic acid that specifically binds with PCAM-1 either alone or in combination with each other and with substances including, but not limited to, ribozymes directed to other proteins (*e.g.*, VEGF-1, MMP-2, and the like), peptidomimetics, small molecules, and drugs (*e.g.*, chemotherapeutic agents), and various permutations thereof as the skilled artisan would determine using methods well-known in the art and methods that are developed in the future with respect to administration of such molecules.

Methods for assessing the level of PCAM-1 (*e.g.*, using anti-PCAM-1 antibodies in Western blot or other immune-based analyses such as ELISA, FACS analysis, or enzyme linked immuno-sandwich assay), methods for assessing the level of PCAM-1 expression in a cell and/or tissues (*e.g.*, using Northern blot analysis, and the like), and/or methods such as Monte Carlo-like DNA/protein binding assays based on detection of binding of a duplex nucleic acid, *e.g.*, PCAM-1 probe 1 (SEQ ID NO:5) and PCAM-1 probe 2 (SEQ ID NO:6), with PCAM-1 (*e.g.*, using nylon membrane-based detection of labeled duplex nucleic acid and/or EMSAs to assess binding of PCAM-1/DNA), are disclosed herein or are well known to those skilled in the art. Such assays can be used to determine the "effective amount" of PCAM-1, nucleic acid, antibody, antisense nucleic acid, ribozyme, recombinant cell, and the like, to be administered to the animal in order to reduce or increase the level of PCAM-1 to a desired level.

C. Methods of diagnosis and assessment of therapies

The present invention includes methods of diagnosis certain diseases, disorders, or conditions (*e.g.*, prostate cancer) which are associated with or mediated by altered and/or mal-expression of PCAM-1.

The invention includes a method of diagnosing a prostate tumor in a previously undiagnosed mammal. The method comprises obtaining a biological sample from the mammal and comparing the level of PCAM-1 (expression, amount, activity) in the sample with the level of PCAM-1 in a sample from an otherwise identical normal mammal that is not afflicted with a prostate tumor. A

higher level of PCAM-1 in the sample from the mammal in question compared with the level of PCAM-1 in the sample obtained from a mammal known not to be afflicted with prostate tumor is an indication that the mammal is afflicted with a prostate tumor. This is because, as disclosed elsewhere herein, an increased level of PCAM-1 expression and/or activity is associated with the presence of prostate cancer. Thus, detection of increased level of PCAM-1 protein, nucleic acid encoding PCAM-1, and/or increased level of PCAM-1 binding with a double-stranded nucleic acid that specifically binds with PCAM-1, in a sample obtained from mammal is an indication that the mammal is afflicted with prostate cancer.

Further, the data disclosed elsewhere herein also demonstrate that there is a correlation between the level of PCAM-1 in the sample and the Gleason Score of the various tumor tissues indicating that the level of PCAM-1 is a staging marker for such tumors. Thus, detection of an altered level of expression of PCAM-1 (whether detected using antibody-based methods or methods based on detection of nucleic acids), or detection of increased PCAM-1 binding of a nucleic acid that specifically binds with PCAM-1 (*e.g.*, such as a nucleic acid having the sequence of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8), is indicative of the stage of a prostate tumor since the data disclosed herewith demonstrates that the level of expression of PCAM-1 is correlated with the Gleason Score of the tumor. Thus, the present invention includes methods of assessing the state, *i.e.*, "staging," a prostate tumor by assessing the level of PCAM-1 in a sample obtained from a mammal compared with the level of PCAM-1 detecting in a sample obtained from an otherwise normal mammal known not to have a prostate cancer tumor or known to have a prostate cancer tumor of a known specific stage and/or having a known Gleason Score.

In one aspect, the biological sample is selected from the group consisting of a blood sample, a prostate biopsy, a urine sample, a prostatic fluid sample, a semen sample, a lymph fluid sample, a seminal vesicle tissue sample, a pleural cavity fluid sample, a perineal cavity fluid sample, a peritoneal cavity fluid sample, a bone marrow sample, a salivary gland fluid sample, and prostate cancer tumor sample, and a sample obtained from other cancer tissues, and the like.

One skilled in the art would understand, based upon the disclosure provided herein, that there are a wide variety of methods for assessing the level of

PCAM-1 in a sample. Such methods include, but are not limited to, antibody-based detection methods (*e.g.*, using anti-PCAM-1 antibodies in Western blot or other immune-based analyses such as ELISA, FACS assay, and enzyme linked immuno-sandwich assay); methods for assessing the level of PCAM-1 expression in a cell and/or tissues (*e.g.*, using Northern blot analysis, and the like), and/or methods such as Monte Carlo-like DNA/protein binding assays based on detection of binding of a duplex nucleic acid, *e.g.*, PCAM-1 probe 1 (SEQ ID NO:5) and PCAM-1 probe 2 (SEQ ID NO:6), with PCAM-1 polypeptide (*e.g.*, using nylon membrane-based detection of double-stranded nucleic acid and/or EMSAs to assess binding of PCAM-1 with a double-stranded nucleic acid that specifically binds with PCAM-1). Thus, methods of detecting PCAM-1, either by detecting a PCAM-1 polypeptide or a nucleic acid encoding PCAM-1 (*i.e.*, RNA or DNA), or a nucleic acid that specifically binds with PCAM-1 polypeptide, are disclosed herein or are well known to those skilled in the art and are encompassed in the present invention. Furthermore, the present invention encompasses similar assays for the detection of a specific protein or nucleic acid in a sample as may be developed in the future.

The invention includes a method of assessing the effectiveness of a treatment for a prostate cancer in a mammal. The method comprises assessing the level of PCAM-1 expression, amount, and/or DNA binding activity, before, during and after a specified course of treatment for a disease, disorder or condition mediated by or associated with increased PCAM-1 expression (*e.g.*, prostate cancer). This is because, as stated previously elsewhere herein, PCAM-1 expression, amount and/or activity is associated with or mediates certain disease states. Thus, assessing the effect of a course of treatment upon PCAM-1 expression/amount/DNA-binding activity indicates the efficacy of the treatment such that a lower level of PCAM-1 expression, amount, or activity indicates that the treatment method is successful.

D. Methods of identifying DNA-binding proteins and their cognate double-stranded oligonucleotide binding partners

The present invention includes methods of identifying DNA-binding proteins and double-stranded oligonucleotides bound by the proteins. The methods comprise contacting a member of a set of semi-random double-stranded

oligonucleotides with a mixture containing DNA-binding proteins. The oligonucleotides are semi-random in that they comprise an unknown random sequence which is flanked on both 5' and 3' sides, but at least two known base pairs. In one embodiment, the oligonucleotide was 8 bp in length where the first basepair was an A then the second basepair was varied with each A, T, G, C, while the flanking known pair was the complementary Watson-Crick basepairing match so that where the nucleotide at position 1 was an "A" the nucleotide at position 8 was "T." Similarly, when the second position was A, the seventh nucleotide was T, and so forth. Thus, a set of semi-random oligonucleotides is generated such that the 2 basepairs at the 5' end and the 2 basepairs at the 3' end are known and there is in between them an unknown core sequence of about 4 basepairs.

The skilled artisan would appreciate, based upon the disclosure provided herein that the known flanking basepairs are not limited to 2. Further, one skilled in the art would understand that the unknown, random, core sequence can range from about 3 to 12 basepairs, such that the double-stranded oligonucleotide preferably ranges in size from about 7 to 16 basepairs, *i.e.*, a 5' end comprising 2 known basepairs followed by 3 to 12 unknown core basepairs, which are in turn followed by 2 known basepairs that are a mirror image of the first 2 known basepairs at the 3' end of the oligomer, where the 2 nucleotides at the 5' end of the molecule would be able to bond with the 2 nucleotides at the 3' end of the molecule according to Watson-Crick basepairing rules such that the first two nucleotides of the oligonucleotide and the last two nucleotides would hybridize with each other if the oligonucleotide was single-stranded and could, but need not, form a short stem and loop structure.

Each semi-random oligonucleotide from the set is then mixed with a mixture comprising DNA-binding proteins. The oligonucleotides and proteins are allowed to incubate under conditions where specific DNA-protein binding can occur. Such conditions are well-known in the art and are exemplified herein and the present invention is not limited to any particular set of reaction conditions. Rather, the present invention includes a wide plethora of reaction conditions well known in the art, disclosed herein, and to be developed in the future, which the skilled artisan, armed with the teaching of the present invention, would understand

could be used to asses the specific binding of a double-stranded nucleic acid with PCAM-1.

The double-stranded oligonucleotide demonstrating the highest binding affinity to DNA-binding proteins is then selected for use in the design of the next probe. More specifically, as depicted in Table 1, the 8 basepair oligonucleotide demonstrating the highest level of binding with a DNA-binding protein mixture (***CANNNNTG**) was selected and a semi-random set of oligonucleotides having this sequence but having an additional known basepair such that the core random sequence was reduced by one basepair to only 3 unknowns (*i.e.*, CACNNNTG, CAGNNNTG, CATNNNTG, and CAANNNTG) was produced. Once again, each member of the set was allowed to bind with a sample comprising DNA-binding proteins and the double-stranded oligonucleotide binding with the highest affinity with the proteins was identified and sequences (*i.e.*, indicated by an asterisk and in bold -- CACNNNTG). This procedure was repeated each time adding a known basepair and decreasing the number of unknown, random core sequence basepairs until the entire sequence of the double-stranded nucleic acid that binds with a DNA-binding protein is identified.

Further, one skilled in the art would appreciate, based upon the disclosure provided herein, that the protein that the double stranded oligonucleotide specifically binds with is also identified using this assay. Indeed, the "Monte Carlo-like" assay of the present invention identified the novel DNA-binding protein PCAM-1, and the novel nucleic acid sequence that binds with the protein (*e.g.*, a nucleic acid having the sequence SEQ ID NO:5 and a nucleic acid having the sequence SEQ ID NO:6). Therefore, the invention encompasses methods of identifying DNA-binding proteins and proteins identified using such methods, including, but not limited to, proteins that are present at a higher level in tumor tissue than in otherwise identical, non-tumor tissue.

The skilled artisan would also appreciate, based upon the disclosure provided herein, that double-stranded oligonucleotides ranging in length from about 7 to 9 basepairs are used because, as more fully set forth elsewhere herein, these are the average lengths of many known DNA sequences that specifically bind with proteins, such as transcription factor proteins involved in the regulation of gene expression (Sambrook et al., 1989, *supra*). Therefore, these lengths were selected

for use in the methods of the present invention. However, the present invention is not limited to these lengths; rather, the invention includes a central, unknown sequence ranging from about 3 to 12 basepairs, flanked by at least 2 known basepair such that the double-stranded oligonucleotide of the invention ranges from about 7 to 16 basepairs in length.

The method further comprises detecting specific DNA-protein binding. The skilled artisan, armed with the teachings set forth herein, would understand that specific DNA-protein binding can be detected using techniques well-known in the art such as those, but not limited to, the techniques exemplified herein, including applying the proteins to a solid support such as a nylon membrane and detecting labeled oligonucleotides that are specifically bound to the membrane to identify the protein that specifically binds with a double-stranded oligonucleotide.

Alternatively, detection of DNA-protein complexes can be performed using electrophoretic mobility shift assays, or EMSAs such as those disclosed herein and/or those known in the art. The protein can be excised from the gel and sequenced to determine the amino acid of the protein that specifically binds a double-stranded oligonucleotide. One skilled in the art, based upon the disclosure provided herein, would understand that the specific detection method for assessing the presence of DNA-protein binding, and for determining the identity (*e.g.*, the amino acid sequence) of the protein, is not crucial and that there are many methods that can be used to detect DNA-protein complexes and to isolate and identify the DNA-binding protein and the double-stranded oligonucleotide bound therewith. Thus, using the methods of the invention, both DNA-binding proteins and the cognate double-stranded oligonucleotides that they bind with can be easily identified and characterized.

The invention also includes a method of identifying DNA-binding proteins and the double-stranded oligonucleotide sequences that they specifically bind with which are associated with a disease, disorder or condition, *e.g.*, prostate cancer. The method comprises identifying DNA-binding proteins and their cognate oligonucleotide binding partners that are present in protein extracts prepared from diseased cells or tissue but which are not detected in protein extracts prepared from otherwise identical protein extracts prepared from normal cells and

tissues known not to have a disease, disorder or condition. Thus, as would be appreciated by the skilled artisan based upon the disclosure provided herein, the methods of the present invention comprise identifying DNA-binding proteins and the oligonucleotides that they bind and to select those DNA-binding proteins and oligonucleotide binding partners that can be detected in protein extracts from diseased tissue but which are not detected, either because the DNA-binding protein is not present, possesses different properties, or is present in an amount beyond the limit of detection of the assay.

Identification of novel DNA-binding proteins the level of which is elevated in diseased but not in normal, non-diseased tissue is important in that such proteins, and their cognate double-stranded oligonucleotides, are potential diagnostic and therapeutic candidates for the diagnosis and treatment of such diseases, disorders or conditions. That is, such DNA-binding proteins are likely to be involved in or be associated with the disease process in that they may regulate cellular processes such as altered expression of certain genes, that are involved in tumorgenesis.

Indeed, PCAM-1, a novel DNA-binding protein identified using the methods disclosed herein, is involved or, at the very least, is associated with prostate cancer such that detection of PCAM-1 in tissue cells and bodily fluids is an effective means for the diagnosis of prostate cancer. In addition, inhibition of PCAM-1 expression in tumor cells (PC-3 ML) and tumor tissues (in SCID mice) decreased the survival of the tumor cells and the survival of the tumors. These results, which are not limited to PCAM-1, demonstrate the importance of identifying DNA-binding proteins, and the DNA that the specifically bind with, associated with a disease, disorder or condition since they are important for the development of diagnostics and therapeutics to treat these diseases, disorders or conditions.

IX. Kits

The invention includes various kits which comprise a compound, such as a nucleic acid encoding PCAM-1, an antibody that specifically binds PCAM-1, a nucleic acid complementary to a nucleic acid encoding PCAM-1 but in an antisense orientation with respect to transcription, a ribozyme that specifically

cleaves PCAM-1 mRNA (*e.g.*, a nucleic acid having sequence SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and the like) and/or compositions of the invention, a nucleic acid that specifically binds with a PCAM-1 polypeptide (*e.g.*, PCAM-1 probe 1 [SEQ ID NO:5] and PCAM-1 probe 2 [SEQ ID NO:6]), an applicator, and instructional materials which describe use of the compound to perform the methods of the invention. Although exemplary kits are described below, the contents of other useful kits will be apparent to the skilled artisan in light of the present disclosure. Each of these kits is included within the invention.

In one aspect, the invention includes a kit for alleviating a disease mediated by mal-expression of PCAM-1. The kit is used pursuant to the methods disclosed in the invention. Briefly, the kit may be used to contact a cell with a nucleic acid complementary to a nucleic acid encoding PCAM-1 where the nucleic acid is in an antisense orientation with respect to transcription to reduce expression of PCAM-1, or with an antibody that specifically binds with PCAM-1, or with a ribozyme that specifically cleaves PCAM-1 mRNA, wherein the decreased expression, amount, or activity of PCAM-1 mediates an beneficial effect. Moreover, the kit comprises an applicator and an instructional material for the use of the kit. These instructions simply embody the examples provided herein.

The kit includes a pharmaceutically-acceptable carrier. The composition is provided in an appropriate amount as set forth elsewhere herein. Further, the route of administration and the frequency of administration are as previously set forth elsewhere herein.

The invention further includes a kit for assessing the effectiveness of an anticancer treatment. The kit comprises a compound that specifically binds with PCAM-1, or a nucleic acid encoding PCAM-1, such that the level of PCAM-1 present in a sample can be assessed. As previously disclosed elsewhere herein, such PCAM-1 detecting compound includes, but is not limited to, an antibody that specifically binds with PCAM-1 (to be used in antibody based detection methods such as, for example, Western blot analysis, enzyme linked immuno-sandwich assay, FACS assay, or ELISA, enzyme immunoassay or EIA, and the like), a nucleic acid that specifically binds with a nucleic acid encoding PCAM-1 (for use in, *e.g.*, Northern and Southern blot analyses), and a duplex nucleic acid that specifically binds with PCAM-1 polypeptide, *e.g.*, PCAM-1 probe 1 (SEQ ID

NO:5) and PCAM-1 probe 2 (SEQ ID NO:6), and double-stranded oligonucleotides that specifically bind with PCAM-1 having the sequence SEQ ID NO:7 and SEQ ID NO:8, all of which can be used to detect PCAM-1 using DNA/protein binding assays (*e.g.*, Monte Carlo-like assay and EMSAs).

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

EXAMPLES

Example 1: Novel assay for identification of DNA binding proteins and the DNA molecules that specifically binds therewith

The experiments presented in this example may be summarized as follows.

The invention relates to the development of a "proteomics" platform for rapid identification of double stranded DNA sequences, which preferentially bind protein(s), expressed in diseased tissue compared with benign or normal tissue from the same patient. The basic approach entails the systematic synthesis of 7 basepair, 8 basepair, and 9 basepair double stranded DNA sequences starting with the degenerate sequences and ending with "completed" sequences (see Table 2, Figure 2). A quantitative "DNA-protein" binding assay on a solid support (*e.g.*, nylon membrane) is employed to assess 'DNA-protein' binding affinity and to identify the DNA sequence(s), which preferentially bind protein(s) from diseased tissue (*i.e.*, cancer), compared to benign or normal tissue from the same patient, *i.e.*, otherwise identical tissue known not to have any detectable disease, disorder or condition.

The invention field relates to identification of novel DNA sequences, which can uniquely bind protein(s) in diseased tissue and/or normal or benign tissue. The invention further relates to the diagnosis, prevention and treatment of

diseases (including cancer) relating to dis-regulation (also referred to malregulation or mal-expression) of nucleic acid expression.

The data disclosed herein demonstrate the discovery of a novel Monte Carlo-like assay for the identification of DNA binding proteins and the DNA molecule that specifically binds with the proteins.

The Materials and Methods used and the Results of the experiments presented in this example are now described.

The present invention discloses a novel rapid "quantitative" screening assay for identification of novel double-stranded oligonucleotides ranging in length from about 7 to 9 basepairs (also referred to as 7 to 9 basepairs) where the oligonucleotides bind a protein in a protein extract prepared using a tissue having a detectable disease, disorder or condition (*i.e.*, cancer), but which oligonucleotides do not detectably bind with proteins obtained from otherwise identical tissue that does not demonstrate a disease, disorder or condition (*i.e.*, normal or benign tissue).

The rationale for this approach is that these DNA sequence lengths represent the average length of known DNA sequences which normally bind transcription factors or co-factors involved in the regulation of gene expression in cells and tissue (Sambrook et al. 1989). Therefore, the "semi-random" screening for DNA sequences which bind protein over expressed in matching tissues from the same patient should identify novel DNA sequences which uniquely bind protein(s) involved in the regulation of gene transcription and gene expression associated with normal, benign or diseased tissue. However, the present invention is not limited to any particular length of oligonucleotide to be interrogated for DNA-binding ability. Thus, although oligonucleotides having a length of about 7 to 9 basepairs are exemplified herein, oligonucleotides having other lengths, including from having a length from about 7 to 16 basepairs (7-16 bps or 7-16 mers), are encompassed in the present invention.

The over-all import of the approach described here resides with the fact that identification of 7 basepair, 8 basepair or 9 basepair sequences (as the 7, 8, and 9 basepair oligonucleotides or oligomers are alternatively referred to herein) associated with a specific pathological condition of the tissue will enable the use of these DNA sequences in the diagnosis of the patient's condition (*i.e.*, as healthy, benign or diseased). That is, DNA-protein binding assays can be performed on

protein obtained from tissue or body fluids to diagnose the patient's condition or disease status or normal status.

The present invention relates to the synthesis of either 7 basepair, 8 basepair or 9-mer double stranded DNA sequences, which selectively bind protein(s) from crude tissue extracts. The DNA sequences are synthesized and (γATP) ^{32}P-radiolabeled and purified by column chromatography according to standard methods of Sambrook et al. (1989). Crude protein extracts were prepared from dissected human tissue of the same patient (*i.e.*, normal, benign and cancer, and tissue not exhibiting in any detectable disease, disorder or condition) and increased amounts of protein (1, 5, 10 and 20 μg total protein) was applied to nylon membrane filters. The nylon membranes were then incubated using a constant amount of radiolabeled double stranded DNA and the filters were washed with phosphate buffer to remove non-specifically bound DNA and protein which failed to bind DNA according to established methods (Sambrook et al., 1987).

Table 1 provides an example of the DNA double stranded sequences (8 basepair) screened using the Monte Carlo-like array protocol disclosed herein. The amount of radiolabeled probe binding to crude protein extracts from different regions of a human prostate (*i.e.*, prostate cancer, benign prostate hyperplasia, and prostate stroma) was compared for each sequence. Background levels (0 or zero) of radiolabeled probe binding to bovine serum albumin were measured for each probe. Routine screening for probes of interest was done in sequential rounds where the initial sequence had a core of 4N (*i.e.*, random sequence). The sequence from this batch with the highest binding level was then used to generate 4 different sequences with a random unknown core sequence of 3N and the binding assays were repeated to identify the sequence with highest binding activity for the tissue site of interest (*i.e.*, cancer in this case). Likewise, the sequence from this batch was subsequently selected and 4 sequences were produced having a core of 2N, 1N and zero N, respectively, useful to identify the sequence with highest binding activity for the tissue site of interest (*i.e.*, cancer).

Once the sequence of interest (*i.e.*, with sequentially increased levels of binding activity for the crude protein extract from prostate cancer) was identified, then experimental testing was carried out to verify the result. The data disclosed in Table 2 demonstrate for example, that the binding activity of a constant

amount of radiolabeled probe (*i.e.*, the(γ-ATP)^{32}P-labeled CACGGATG probe (1 ng at 100,000 cpm)) increased with increased amounts of crude protein (10 μg) from prostate cancer tissue spotted on a nylon membrane filter. The amount of probe binding to benign prostate hyperplasia, normal stroma and bovine serum albumin did not increase in comparable studies, however (Table 2). A degenerate probe (CANNNNTG) used as a control in these comparative assays failed to bind protein at levels above background levels of CACGGATG binding to bovine serum albumin (*i.e.*, non-specific binding). Positive control studies with a known probe which normally binds AP-2 confirmed that all the protein extracts were "good" and confirmed and that differences in the results were not attributable to how the protein was prepared or the methods used in the binding assays.

Finally, the selective binding of the probe identified was compared for comparable tissue extracts from multiple patient prostates (n=11) in order to verify the observation. The data disclosed herein confirm that the screening and selection strategy was successfully reduced to practice (Table 3).

Likewise, the data demonstrate that a specific double stranded DNA probe consistently bound a protein(s) associated with prostate cancer (Gleason Score 6-8). Figure 2 depicts an example of data from a screening assay demonstrating the basic utility of the "DNA-Proteomics" platform for measuring ^{32}P-labeled double stranded DNA oligonucleotide (approximately 8 basepair) (CACGGATG [SEQ ID NO:5]) binding to a specific protein (PCAM-1) in crude protein extracts. The ^{32}P-labeled probe failed to bind protein in seminal vesicle and BPH or HGPIN preparations (Figure 2, lanes 1-3) (or bound a protein faintly in the HGPIN extracts), but strongly bound PCAM-1 expressed in prostate cancer tissue extracts from glands of Gleason score 8, 7 and 5 in lanes 4-6, respectively. In addition, the probe bound PCAM-1 expressed in a lambda phage clone expressing PCAM-1 protein (lane 7, a-c) and in crude protein extracts of the PC-3 ML cells (lane 7, d-f).

An identical strategy and approach can be undertaken for the 7 basepair and 9 basepair sequences. The difference would be to start with core sequences of 3N and 5N, respectively.

TABLE 1

Screening of 8-mer oligonucleotide sequences

Radiolabeled Probe	Prostate Cancer	Benign Prostate Hyperplasia	Prostate Stroma	Bovine Serum Albumin
AANNNNTT	0	0	0	0
ATNNNNAT	+1	0	0	0
AGNNNNCT	+1	+1	+1	0
ACNNNNGT	+1	+1	+1	0
TANNNNTA	0	0	0	0
TTNNNNAA	0	0	0	0
TGNNNNCA	0	0	0	0
TCNNNNGA	0	0	0	0
GANNNNTC	+2	+2	+2	0
GTNNNNAC	+1	+1	+1	0
GGNNNNCC	+1	+1	+1	0
GCNNNNGC	+1	+1	+1	0
***CANNNNTG**	+3	+1	+1	0
CTNNNNAG	+1	0	0	0
CGNNNNCG	+1	+1	+1	0
CCNNNNGG	+1	+1	+1	0
***CACNNNTG**	+3	+1	+1	0
CAGNNNTG	+1	+1	+1	0
CATNNNTG	+1	+1	+1	0
CAANNNTG	+1	0	0	0
***CACGNNTG**	+4	+1	+1	0
CACCNNTG	+2	+1	+1	0
CACTNNTG	+1	+2	+2	0
CACANNTG	+3	+1	+1	0
***CACGGNTG**	+5	+1	+1	0
CACGCNTG	+1	+2	+2	0
CACGTNTG	+2	+1	+1	0
CACGANTG	+2	+1	+1	0
***CACGGATG**	+6	0	0	0
CACGGTTG	+3	+1	+1	0
CACGGGTG	+2	+1	+1	0
CACGGCTG	+2	+1	+1	0
***CACGGATG**	+6	0	0	0

Aliquots of the nuclear protein extracts (5, 10 and 20 μg protein total in triplicate test wells for each protein concentration) are dotted on Nylon membrane filters and incubated with the $(\gamma\text{-ATP})^{32}$P-labeled probe (1 nanogram at 100,000 counts per minute). Values are averaged for the triplicate measurements and then normalized for 10 μg protein from measurements of DNA binding to the 3 different protein concentrations tested. Control assays with a $(\gamma\text{-ATP})^{32}$P-labeled AP-2 binding probe (1 ng at 100,000 cpm) provide control measurements validating the quality of the protein extracts prepared and usually yielded counts of 100-5000 cpm (+1). The numbers represent: (0) <1000; (+1) 1000-5000; (+2) 5001-10,000; (+3) 10,001-20,000; (+4) 20,001-30,000; (+5) 30,001-40,000; (+6) 40,001-50,000 cpm. N = A,T,G,C.

TABLE 2

Measurements of **CACGGATG** binding to crude protein extracts (5/10/20 µg protein, respectively*).

Probe	Prostate Cancer	Benign Prostate Hyperplasia	Prostate Stroma	Bovine Serum Albumin
CACGGATG	+3/+6/+13	0/+1/+1	0/0/+1	0/0/0
CANNNNTG	0/+1/+1	0/0/+1	0/0/0	0/0/0
AP-2 Probe	+1/+1/+1	+1/+1/+1	+1/+1/+1	0/0/0

See legend for Table 1 for methods.

TABLE 3

Measurements of CACGGATG binding to crude protein extract (10 µg protein) from 11 different prostates.

Prostate Specimen	Probe	Prostate Cancer	Benign Prostate Hyperplasia	Bovine Serum Albumin
1	**CACGGATG**	+5	0	0
1	CANNNNTG	+1	0	0
1	AP-2 Probe	+1	+1	0
2	**CACGGATG**	+6	+1	0
2	CANNNNTG	+1	+1	0
2	AP-2 Probe	+1	+1	0
3	**CACGGATG**	+5	0	0
3	CANNNNTG	+1	+1	0
3	AP-2 Probe	+1	+1	0
4	**CACGGATG**	+5	0	0
4	CANNNNTG	+1	+1	0
4	AP-2 Probe	+1	+1	0
5	**CACGGATG**	+6	+1	0
5	CANNNNTG	0	0	0
5	AP-2 Probe	+1	+1	0
6	**CACGGATG**	+7	+1	0
6	CANNNNTG	0	+1	0
6	AP-2 Probe	+1	+1	0
7	**CACGGATG**	+6	+1	0
7	CANNNNTG	0	+1	0
7	AP-2 Probe	+1	+1	0
8	**CACGGATG**	+5	+1	0
8	CANNNNTG	0	+1	0
8	AP-2 Probe	+1	+1	0
9	**CACGGATG**	+6	+1	0
9	CANNNNTG	+1	0	0

9	AP-2 Probe	+1	+1	0
10	**CACGGATG**	+5	0	0
10	CANNNNTG	+1	+1	0
10	AP-2 Probe	+1	+1	0
11	**CACGGATG**	+6	+1	0
11	CANNNNTG	+1	0	0
11	AP-2 Probe	+1	+1	0

See legend for Table 1 for methods.

Example 2: Identification of a novel DNA binding protein, PCAM-1, and the DNA molecules that specifically bind therewith

The experiments presented in this example may be summarized as follows.

The data disclosed herein demonstrate the discovery of a novel nucleic acid encoding a prostate cancer marker protein and a novel DNA molecule that specifically binds with the protein. These nucleic and amino acid sequences can be used to detect prostate cancer.

This invention relates to nucleic acid and amino acid sequences of DNA consensus domains which, bind a novel marker protein for cancer, herein referred to as "PCAM-1" protein. The invention further relates to the use of these sequences and probes which specifically recognize the PCAM-1 protein in the diagnosis, prevention and treatment of diseases related to disregulated cell growth and proliferation and cancer.

The Materials and Methods used in the experiments presented in this example are now described.

A novel "Monte Carlo-like" type screening assay for identification of novel DNA binding proteins (*i.e.*, transcription factors involved in chromosomal recombination) in nuclear extracts derived from dissected human prostate tissues was developed. For testing of oligonucleotides of 8 basepairs in length, each member of a set of stranded DNA sequences (n=4096 combinations as depicted in Table 1) was screened individual to assess protein binding by the oligonucleotide on nitrocellulose filters and in electrophoretic mobility gel shift assays (EMSAs).

Scintillation counting and phosphoimaging revealed that nuclear protein(s) from prostate cancer glands specifically bound a novel DNA sequence (CACGGATG [SEQ ID NO:5]), designated as "PCAM-1 probe 1." The

CACGGATG sequence was very similar to known break point cluster region sequences (Rabbitts and Boehm, 1991, Advances in Immunology 50:119-146) associated with chromosomal breakage in T-cells and B-cells.

In addition, the data disclosed herein also demonstrate that another double-stranded oligonucleotide (CACAATGA [SEQ ID NO:6]), designated "PCAM-1 probe 2", also bound specifically with PCAM-1. Thus, double-stranded oligonucleotides that specifically bind with PCAM-1 include the following:

"PCAM-1 probe 1" (SEQ ID NO:5):

5' - CACGGATG - 3'
3' - GTGCCTAC - 5'

"PCAM-1 probe 2" (SEQ ID NO:6)

5' - CACAATGA - 3'
3' - GTGTTACT - 5'

Utilization of a double stranded CACGGATG probe to screen cDNA libraries identified phagemid clones which expressed the "PCAM-1" protein. The recombinant protein was found to bind the presumptive CACGGATG (SEQ ID NO:5) and other known break point cluster region sequences (Rabbitts and Boehm, *ibid.*) in EMSAs. EMSAs and ELISAs demonstrated that the over-expression of PCAM-1 protein in urine and serum was diagnostic and prognostic for human prostate cancer.

PCAM-1 is at least about 97% homologous with the S2-ribosomal protein, and exhibiting at least 5 specific base pair mutations which render the PCAM-1 protein distinct from S2. In comparison, S2 is a totally different protein which is part of the ribosomal complex in normal cells and which does not demonstrate any DNA binding activity. It should be noted that there are several reports demonstrating a connection between over-expression of genes encoding ribosomal proteins and cancer (Chiao et al., 1992, Mol. Carcinog. 5:219-231; Fernandez-Pol et al., 1993, J. Biol. Chem. 268: 21198-211204; Fernandez-Pol et al., 1994, Cell Growth & Differentiation 5:821-825; Fernandez-Pol, 1996, Anticancer Res. 16:2177-2186; Chan et al., 1996, Biochem. and Biophys. Res. Comm. 228:141-147; Chan et al., 1996, Biochem. and Biophys. Res. Comm. 225:952-956; Wool, 1996, Trends in Biochemical Sciences 21:164-165.; Wool, 1997, In: The

ribosomal RNA and Group I introns, pp. 153-178, Green and Schroeder, eds., R.G. Landes Co., Austin, TX; Wool et al., 1995, Biochemistry & Cell Biology 73:933-947; Vaarala et al., 1998, Int. J. Cancer 78:27-32), indicating that increased numbers of ribosomes and protein synthesis are associated with the disease status.

For example, Northern blotting studies with the normal S2 mRNA revealed that the S2 mRNA was elevated in head and neck cancer, but barely detectable in normal tissue (Chaio and Tainsky (1992, Mol. Carcinog. 5:219-231). Without wishing to be bound by any particular theory, the wide spread belief is that ribosomal proteins might somehow play an important role in elevating protein synthesis in cancer (Chiao et al., 1992, Mol. Carcinog. 5:219-231; Fernandez-Pol et al., 1993, J. Biol. Chem. 268: 21198-211204; Fernandez-Pol et al., 1994, Cell Growth & Differentiation 5:821-825; Fernandez-Pol, 1996, Anticancer Res. 16:2177-2186; Chan et al., 1996, Biochem. and Biophys. Res. Comm. 228:141-147; Chan et al., 1996, Biochem. and Biophys. Res. Comm. 225:952-956; Wool, 1996, Trends in Biochemical Sciences 21:164-165.; Wool, 1997, In: The ribosomal RNA and Group I introns, pp. 153-178, Green and Schroeder, eds., R.G. Landes Co., Austin, TX; Wool et al., 1995, Biochemistry & Cell Biology 73:933-947; Vaarala et al., 1998, Int. J. Cancer 78:27-32).

One alternative possibility, without wishing to be bound by any particular theory, is that a putative "leucine zipper" sequence motifs or mutant motifs, characteristic of numerous ribosomal proteins, might be mutated and that the mutated "leucine zipper" domain can then bind to nucleic acids (Fernandez-Pol, 1996, Anticancer Res. 16:2177-2186, Wool, 1996, Trends in Biochemical Sciences 21:164-165.; Wool, 1997, In: The ribosomal RNA and Group I introns, pp. 153-178, Green and Schroeder, eds., R.G. Landes Co., Austin, TX) and either function as a DNA binding protein, a nuclease, control ligation or regulate gene transcriptional and translational in cancer cells. For example, the rat ribosomal protein S3a is identical to the product of the rat v-fos transformation effector gene (Chan et al., 1996, Biochem. and Biophys. Res. Comm. 228:141-147). S3a is involved in initiation of protein synthesis and is also related to proteins involved in the regulation of growth and the cell cycle (Chan et al., 1996, Biochem. and Biophys. Res. Comm. 228:141-147). Likewise, the rat ribosomal protein L10 is homologous to a DNA-binding protein and to a putative Wilm's tumor suppressor

gene (Chan et al., 1996, Biochem. and Biophys. Res. Comm. 225:952-956). In sum, these studies suggest that mutant "ribosomal-like" proteins might be prognostic or diagnostic for cancer and play important roles in regulating chromosomal DNA activities, gene expression, and the behavior of cancer cells.

It should be understood that the present proteins, nucleotide sequences and methods described in this invention are not limited to the particular methodology, protocols, cell lines, vectors, reagents and applications described. These may vary. Likewise, it should be understood that the terminology used herein is strictly for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention or applications. The scope of the invention is only limited by the appended claims.

Development of markers for the early detection of cancers such as prostate cancer is essential to improved treatment of cancer. With respect to prostate cancer, it is generally believed that serum prostate specific antigen (PSA) levels are neither sensitive nor specific for identification of patients with prostate cancer (Garnick, M.B. and Fair, W.R. Prostate Cancer. Scientific American, December 1998, 75-83). It has been estimated that only about 25% of men with prostate cancer are detected at serum PSA levels ranging from >4 ng/ml to 10 ng/ml (*i.e.*, false negatives). Likewise, as many as 30% of men with benign prostate hyperplasia have elevated PSA levels (*i.e.*, false positives). In addition, attempts to confirm the diagnosis with biopsies are only successful in 10% to 15% of the patients. Thus, development of more sensitive and more specific assays for cancers, including prostate cancer, is clearly needed. Non-invasive and inexpensive urine based screening assays, which would enable implementation through mass community screening programs, or in routine clinical examinations would be particularly useful.

The present invention relates to nucleic acid sequences, which can be used in screening assays to identify novel DNA binding proteins in nuclear extracts derived from human tissues. In one embodiment, the screening assay is useful in identifying novel transcription factors over-expressed in nuclear protein extracts of prostate tissue (*i.e.*, glands). For this assay, 8 basepair double stranded DNA probes (n=4096) were designed. The DNA probes were then used to screen for differences in protein-DNA binding affinity among matched protein extracts

from cancer, benign, high grade prostatic intraepithelial neoplasia, and seminal vesicle tissue in matched specimens from the same patient. Binding of the proteins was determined via nitrocellulose filters and electrophoretic mobility gel shift assays (EMSAs).

Scintillation counting and phosphoimaging revealed that proteins isolated from nuclear extracts of advanced human prostate cancer tissues specifically bound a nucleic acid sequence comprising CACGGATG. Protein extracts from other tissues examined failed to bind this nucleic acid sequence. This sequence is similar to known BPCR sequences (Rabbitts and Boehm, 1991, Advances in Immunology 50:119-146) associated with chromosomal breakage in T-cells and B-cells.

The specific DNA sequence (CACGGATG) identified was employed to screen cDNA libraries developed from PC-3ML prostate cells (Wang et al., 1998, Oncology Research 10:219-233, 1998). This screening resulted in the identification of phagemid clones, which expressed a PCAM-1 protein. Subcloning of the PCAM-1 gene showed that this gene exhibits approximately about 97% homology with the chromosomal protein S2 and LLRep3. A nucleic acid sequence encoding this PCAM-1 protein (SEQ ID NO:1) is depicted in figure 1 along with the deduced amino acid sequence of this polypeptide (SEQ ID NO:2). This recombinant PCAM-1 protein was demonstrated to bind to the putative BPCR regions and known BPCRs in EMSAs.

The data disclosed herein demonstrate that "DNA-protein" binding assays utilizing EMSAs or nylon filter based binding assays have been developed for the identification of PCAM-1 in biological samples. Using EMSAs, PCAM-1 was detected in tissue extracts, and in urine and serum from human patients.

Polyclonal and monoclonal antibodies (*i.e.*, IgG antibodies) were generated in rabbits and mice, respectively, using standard methods familiar to those schooled in the art of producing antibodies utilizing purified recombinant protein, in this case, PCAM-1, as an antigen.

Antibodies were characterized as being specific for the 33 kDa PCAM-1 antigen using Western blotting analysis, EIAs and immunostaining techniques according to standard methods familiar to those schooled in the art. The results disclosed herein demonstrate that the PCAM-1 antibody specifically

recognizes PCAM-1 in recombinant plasmid protein extracts, in prostate tumor cell protein extracts and cells, and in urine and serum samples obtained from patients with prostate cancer.

Further, EIAs with PCAM-1 specific antibodies demonstrated that the protein was a highly sensitive tissue marker for prostate cancer. As shown in Table 4, PCAM-1 is a significantly better prognostic and diagnostic marker for prostate cancer compared with PSA in prostate tissue extracts. In these experiments, nuclear protein extracts from microdissected regions of the prostate (n=40 radical prostatectomies examined) expressed significantly elevated levels of PCAM-1 compared to very low levels detected in matching seminal vesicle (SV), benign prostatic hyperplasia (BPH) or high grade prostatic intraepithelial neoplasm (HGPIN) foci.

Further, the data disclosed herein demonstrate that the amounts of PCAM-1 (μg/mg DNA) increased as a function of the Gleason Score (GS) as described by Gleason et al., 1993, J. Urol. 149: 1568-1576).

In comparison, PSA levels (μg/mg DNA) were elevated in BPH, HGPIN, and GS specimens, but were significantly reduced in tissue extracts from GS 6, GS 7, and GS 8-10 foci. As disclosed in Table 4, the PSA levels in the tissue extracts were inversely proportional to the serum PSA levels (ng/ml) detected prior to prostatectomy. Serum PSA levels increased as a function of the Gleason Score (Table 4).

TABLE 4

PCAM-1 and PSA in microdissected tissues.

Pathology	#Tissue PCAM-1	#Tissue PSA	Serum PSA
SV (n=30)	0	0	NA
SM (n= 5)	0	0	NA
BPH (n=24)	0	6.2 ± 0.7	NA
HGPIN (n= 6)	0.1 ± 0.03	3.1 ± 0.3	NA
GS 4 (n= 8)	1.8 ± 0.31	1.8 ± 0.31	5.5 ± 0.6 (5.5-12.8)*
GS 6 (n=13)	10.5 ± 1.15	0.5 ± 0.05	13.8 ± 7.9 (6.1-18.9)*
GS 7 (n=10)	20.3 ± 2.06	0.3 ± 0.02	11.5 ± 4.6 (8.9-43.3)*
GS 8-10 (n= 9)	25.2 ± 3.31	0.2 ± 0.01	15.5 ± 5.6 (9.5-87.0)*

Note that following radical prostatectomy (n=40 total), the different glandular foci and tissues were dissected from sagittal sections of the prostates. All BPH and

HGPIN specimens came from the same prostates exhibiting cancer. Samples were assayed at least 3 times and the data averaged for all the patients in the cohort studied. *(range of PSA detected). #PCAM-1 and PSA levels (μg/mg DNA). NA – not applicable. All serum PSA measurements were from routine diagnostic tests taken upon examination of the patient by the Urologist and prior to radical prostatectomy.

Diagnostic tests were conducted to compare urine PCAM-1 levels with serum PSA levels in patients. Data from these tests are disclosed in Table 5.

TABLE 5
PCAM-1 urine assay (n=213 total)

Diagnosis	*PCAM-1 Positive	*PCAM-1 Negative
Prostate Cancer Biopsy positive: (GS 4-8)	24/33(6-13 ng/ml)	9/33
Post-Radical Prostatectomy	2/14 (5-7 ng/ml)	12/14 (<1 ng/ml)
BPH	15/96 (2-8.3 ng/ml)	81/96 (0.4-12 ng/ml)
Other Prostatic Disorders	1/14	13/14
Erectile Dysfunctions	2/13	11/13
Volunteers (22-53 yrs)	0/40	40/40
Women	1/5(neurogenic bladder)	4/5
Renal Cancer	1/1	0/1
Rectal Cancer	0/2	2/2
Infections/Inflammation	5/9	4/9

Detection limit cut offs were: *PCAM-1 positive (>0.2 ng/ml); *PCAM-1 negative (<0.2 ng/ml). The PCAM-1 levels ranged from 0.2-93 ng/l in PCAM-1 positive patients; and from 0-0.2 ng/ml in PCAM-1 negative patients.

The EIA studies on human urine were carried out with freshly collected urine or urine stored frozen according to methods familiar to those schooled in the art. In brief, the urine sample (100-200 μl) was applied to 96 well titer plates, the antigen allowed to attach for several hours, the plates washed with buffer, primary and secondary antibody were applied, and antibody detecting reagents were added, and the plates were read in a MicroTiter Plate ELISA reader (set at A450 nm) (BioRad, Hercules, CA).

As shown in Table 5, the sensitivity of the urine PCAM-1 assay was 73% (*i.e.*, n=24/33) and correlated with the patients having elevated serum PSA levels and biopsy positive specimens (GS 4-8). Interestingly, in 2 patients with their prostates removed 3-4 years before the assay (*i.e.*, GS 8-10, stage T3 cancers),

the urine PCAM-1 levels were elevated and these patients also had elevated serum PSA levels (*i.e.*, greater than about 5 ng/ml). These patients are currently under observation to determine whether there is recurring cancer. Conversely, 12 of these patients which were negative for urine PCAM-1 (*i.e.* GS 5-6, stage T2 cancers) and they also had very low serum PSA values (<1 ng/ml).

In patients diagnosed with BPH (and no indication of cancer), about 16% (n=15/96) exhibited elevated PCAM-1 urine. Several (n=3/96) also had elevated serum PSA levels. In this cohort of patients, 84% (n=81/96) of the BPH patients were negative for PCAM-1. In these specimens n=40/96 (about 42%) also had low serum PSA levels (*i.e.* less than about 2 ng/ml). Of the 40 volunteer men, all were negative for PCAM-1 and had low serum PSA levels. Presumably, they were also negative for PSA. Interestingly, 1 patient with rectal cancer was positive for PCAM-1 and 5 patients (n=5/9) with infections or inflammation were positive for PCAM-1, indicating false positives might arise from infections or inflammation.

Thus, these data demonstrate that the sensitivity of the PCAM-1 urine assay is about 73% for prostate cancer. The overall specificity (*i.e.*, total negative divided by total patients without the disease) was 167/180 or about 92%. Accordingly, the data disclosed herein demonstrate that PCAM-1 protein can be an independent diagnostic marker for cancer and, in particular, prostate cancer.

The present invention also relates to the screening assay and 8 basepair nucleic acid sequences identified, which, are capable of detecting PCAM-1. In one embodiment, the nucleic acid is a probe comprising the nucleic sequence of SEQ ID NO:5, and the sequence SEQ ID NO:6. Further, the double-stranded oligonucleotides having the sequence SEQ ID NO:7 and SEQ ID NO:8, also bind specifically with PCAM-1 and can be used to detect and assess the level of PCAM-1.

In one aspect of the present invention, nucleic acid sequences which encode the PCAM-1 protein (SEQ ID NO:1), and the amino acid sequence of the PCAM-1 protein encoded by these nucleic acid sequences (SEQ ID NO:2) are provided. An exemplary nucleic acid sequence encoding the PCAM-1 protein (SEQ ID NO:1) and an exemplary deduced amino acid sequence (SEQ ID NO:2) is depicted in Figures 1A and 1B, respectively.

"Nucleic acid sequence" as used herein refers to an oligonucleotide,

nucleotide, or polynucleotide and fragments or portions thereof and to DNA or RNA or genomic or synthetic origin which may be single- or double-stranded and represent the sense or anti-sense strand. The terms "amino acid sequence"; "polypeptide" or "protein" as used herein refers to an amino acid sequence of a naturally occurring protein molecules associated with the recited protein molecule.

The present invention also relates to expression vectors and host cells containing expression vectors which comprise these nucleic acid sequences. Expression vectors and host cells, which can be transfected with an expression vector, are well known in the art. Methods for incorporating a selected nucleic acid sequence such as that of the present invention into a vector and ultimately into a host cells are also well known.

The nucleic acid and amino acid sequences of the present invention are useful in developing screening assays for detection of PCAM-1 protein in biological samples. As demonstrated herein, in one embodiment, antibodies can be raised against the PCAM-1 protein and used in an immunoassay such as an EIA or ELISA to detect PCAM-1 protein in a biological sample such as tissue, sputum, urine or serum. Antibodies can be raised against this protein in accordance with well known procedures. Alternatively, labeled nucleic acid probes can be prepared from the nucleic acid sequences of the present invention and used in EMSAs to detect PCAM-1 in nuclear extracts of tissue biopsy samples.

Thus, another aspect of the present invention relates to methods and kits for detection of PCAM-1 in biological samples. As demonstrated herein, detection of PCAM-1 levels in a biological sample of a patient is useful in diagnosing and prognosticating prostate cancer or other cancers in the patient. In the method of the present invention a biological sample is obtained from a patient and then contacted with a means for detecting PCAM-1 in the biological sample. In one embodiment, this means the kit can comprise an antibody raised against the PCAM-1 protein, which is capable of detecting PCAM-1 protein in biological samples such as tissue, sputum, urine and serum. In another embodiment, this means the kit can comprise a labeled nucleic acid probe such as CACGGATG, which is capable of detecting PCAM-1 protein in biological samples such as tissue biopsies.

Accordingly in the kits of the present invention a means for

detecting PCAM-1 protein in a sample and a PCAM-1 protein standard is provided. Means for detecting PCAM-1 protein may comprise an antibody raised against the PCAM-1 protein or a labeled nucleic acid probe capable of binding to the protein. The presence of PCAM-1 in the biological sample is indicative of the patient having prostate cancer. Methods and kits of the present invention can also be used in patients with prostate cancer to assess their prognosis and evaluate treatments by monitoring changes in levels of PCAM-1 in the patient over time. Increases in the level of PCAM-1 over time is indicative of the cancer progressing while decreases in the level of PCAM-1 over time is indicative of regression of the cancer.

Further, it is believed that these methods and kits for detecting PCAM-1 protein levels may also be useful in diagnosing and prognosticating other types of cancer, inflammatory conditions, infections and genetic mutations.

Example 3: Modulation of PCAM-1 expression using enzymatic nucleic acids and treatment of diseases, disorders or conditions associated with PCAM-1 expression

The experiments presented in this example may be summarized as follows.

The prostate cancer marker 1 (PCAM-1) is a 33 kDa cytoplasmic protein which, as more fully disclosed elsewhere previously herein, is over-expressed in human prostate cancer tissue and is detected in the urine of patients afflicted with prostate cancer. Without wishing to be bound by any particular theory, expression of PCAM-1 can convey a selective growth and/or survival advantage to tumor cells and/or cause chromosomal alteration(s) which lead to the development of prostate cancer or other cancers. Consequently, therapeutic strategies based on modulation of PCAM-1 expression, which can potentially inhibit or reduce the aberrant expression of PCAM-1, were examined as potential anti-cancer therapies. The data disclosed herein demonstrate the use of a PCAM-1 specific ribozyme, designated PCAM-1 ZYM-1, to inhibit PCAM-1 expression and the therapeutic effects related thereto.

The Materials and Methods used in and the Results of the experiments presented in this example are now described.

Selection of PCAM-1 DNA ZYM Cleavage Site in Human PCAM-1 RNA

Targets for useful ribozymes can be determined as disclosed in Draper et al., WO 93/23569; Sullivan et al., WO 93/23057; Thompson et al., WO 94/02595; Draper et al., WO 95/04818; McSwiggen et al., U.S. Pat. No. 5,525,468, and hereby incorporated by reference herein in totality. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be optimized and delivered as described therein.

To test whether the sites predicted by the computer-based RNA folding algorithm corresponded to accessible sites in PCAM-1 RNA, 2 hammerhead sites were selected for analysis. PCAM-1 DNA ZYM target sites were chosen by analyzing genomic sequences of human PCAM-1 and prioritizing the sites on the basis of folding.

Hammerhead PCAM-1 DNA ZYMs were designed that could bind each target and were individually analyzed by computer folding (Christoffersen et al., 1994 J. Mol. Struc. Theochem. 311:273; Jaeger et al., 1989, Proc. Natl. Acad. Sci. USA, 86:7706; Jaeger et al., 1989, RNA 2:419-428) to assess whether the PCAM-1 DNA ZYM sequences fold into the appropriate secondary structure. The PCAM-1 DNA ZYMs with unfavorable intramolecular interactions between the binding arms and the catalytic core were eliminated from consideration. As noted below, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Chemical Synthesis and Purification of PCAM-1 DNA ZYMs for Efficient Cleavage of PCAM-1 RNA

PCAM-1 DNA ZYMs of the hammerhead or hairpin motif were designed to anneal to the 5' region in the RNA message (*i.e.*, 1-31 bp). The binding arms are complementary to the target site sequences described above. The PCAM-1 DNA ZYMs were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described in Usman et al., (1987 J. Am.

Chem. Soc., 109, 7845), Scaringe et al., (1990 Nucleic Acids Res., 18, 5433) and Wincott et al., *supra*, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were greater than about 98%. Hairpin PCAM-1 DNA ZYMs were synthesized in two parts and annealed to reconstruct the active PCAM-1 DNA ZYMs as previously described for ribozymes (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). All PCAM-1 DNA ZYMs were modified to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992, TIBS 17: 34). PCAM-1 DNA ZYMs were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; See Wincott et al., *supra*; the totality of which is hereby incorporated herein by reference) and were resuspended in water. The general structures of the chemically synthesized PCAM-1 DNA ZYM-1 used in this study are shown in Figures 3 and 4 and the sequences of the various PCAM-1 ZYMs exemplified herein are SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11.

PCAM-1 DNA ZYM-1 DNA enzyme Cleavage of PCAM-1 RNA Target

A hammerhead-type PCAM-1 DNA ZYM-1 targeted to the human PCAM-1 RNA were designed and synthesized to test the cleavage activity *in vitro*. The target sequences and the nucleotide location within the PCAM-1 mRNA are given in Figure 3. All hammerhead PCAM-1 DNA ZYMs were synthesized with binding arms (Stem I and II) lengths of seven nucleotides. The relative abilities of a Hammerhead PCAM-1 DNA ZYM 1 to cleave human PCAM-1 RNA is summarized in Figures 6-8.

Full-length or partially full-length, internally-labeled target RNA for PCAM-1 DNA ZYM 1 cleavage assay was prepared by *in vitro* transcription in the presence of [alpha ^{32}P] CTP, passed over a G-50 Sephadex column using spin chromatography, and then used as substrate RNA without further purification. Alternatively, substrates were 5'-^{32}P-end labeled using T4 polynucleotide kinase enzyme.

Assays were performed by pre-warming a 2X concentration of

purified PCAM-1 DNA ZYM 1 in DNA enzyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM $MgCl_2$) and the cleavage reaction was initiated by adding the 2 times PCAM-1 DNA ZYM 1 mix to an equal volume of substrate RNA (maximum of 1-5 nM) that was also pre-warmed in cleavage buffer.

As an initial screen, assays were carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 μM PCAM-1 DNA ZYM-1 (*i.e.*, PCAM-1 DNA ZYM-1 excess). The reaction was quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample was heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by PCAM-1 DNA ZYM-1 cleavage were visualized on an autoradiograph of the gel. The percentage of cleavage was determined by Phosphor Imager™ quantitation of bands representing the intact substrate and the cleavage products.

Single Turnover Reaction

Alternatively, cleavage reactions were carried out in 50 mM Tris-HCl, pH 7.5 and 10 mM $MgCl_2$ at 37°C. I n order to disrupt aggregates that can form during storage, unlabeled PCAM-1 DNA ZYMs and 5' end labeled substrate were denatured and renatured separately in standard cleavage buffer (50 mM Tris-HCl, pH 7.5) by heating to 90°C for 2 minutes and then they were allowed to equilibrate to the reaction temperature of 37°C for 15 minutes. Each RNA solution was then adjusted to a final concentration of 10 mM $MgCl_2$ and incubated at 37°C for an additional 15 minutes.

Cleavage reactions were initiated by combining the PCAM-1 DNA ZYM 1 and the substrate samples to the required concentrations in a final volume of 100 μl PCAM-1 DNA ZYM 1 concentration was 40 nM and substrate concentration was 1 nM. The reaction was also repeated using double (2 nM) and half (0.5 nM) the concentration of substrate to verify that the reaction was indeed performed under single turnover conditions. Aliquots of 10 μl were removed at appropriate time intervals between 0 and 120 minutes and quenched by adding an equal volume of formamide loading buffer (9:1 (v:v) formamide:1X TBE) and frozen on dry ice.

Product and substrate were separated using denaturing 20%

polyacrylamide (7M urea) gel electrophoresis. To determine the fraction of cleavage, substrate and product bands were located by autoradiography of wet gels and quantified by densitometry of these autoradiograms. The autoradiograms were scanned using an Molecular Dynamics, Inc. (San Diego, CA), focus scanner connected to a PC computer and the images were saved as TIFF files. The programmed NIH Image 1.58 (Division of Computing and Research Technology, NIH, Bethesda, MD) was used to plot and quantify the band intensities. In addition, the relevant bands were excised from the gel and were quantified by scintillation counting of the slices cut from the gel using a Packard Tricarb 2000 CA liquid scintillation analyzer (Fisher Sci. Co., Pittsburgh, PA).

Reaction rate constants (k) were obtained from the slope of semi-logarithmic plots of the amount of substrate remaining versus time. The activity half time $t_{1/2}$ was calculated as 0.693/k. Each rate constant was determined from duplicate experiments.

In order to assess the specificity of cleavage demonstrated under the above conditions, each experiment was repeated using a different substrate, relating to another site along the human PCAM-1 mRNA. All conditions remained as described above except samples were taken over a longer time period, *i.e.*, aliquots were examined at intervals spanning over 24 hours rather than over 2 hours.

Multiple Turnover Reactions

The kinetic characteristics of PCAM-1 DNA ZYM-1 were determined from Eadie-Hofstee plots obtained from initial velocities with multiple turnovers done with 5' ^{32}P labeled substrate.

Cleavage reactions were carried out in 50 mM Tris.HCl, pH 7.5 and 10 mM $MgCl_2$ at 37°C. Stock solutions of 100 nM PCAM-1 DNA ZYM 1 and 500 nM-2 μM substrate RNA were prepared in 50 mM Tris-HCl, pH 7.5, preheated separately at 90°C for 2 minutes and cooled to 37°C for 15 minutes. After $MgCl_2$ was added to each of these solutions to a final volume of 10 mM, a further incubation period of 15 minutes at 37_oC was performed.

Cleavage reactions were performed in a final volume of 100 μl with a concentration of 10 nM PCAM-1 DNA ZYM-1 and concentrations of substrate between 100 nM and 1 μM. Reactions were initiated by the addition of PCAM-1

DNA ZYM-1 stock solution to substrate. Aliquots of 10 µl were taken at time intervals between 0 and 120 minutes, quenched by adding an equal volume of formamide loading buffer and frozen on dry ice. Intact substrate and products of cleavage were separated by electrophoresis on a 20% polyacrylamide/7M urea denaturing gel and were detected by autoradiography. The degree of cleavage at each time point was quantified using scanning densitometry of the resulting autoradiogram. Initial rates of reaction were measured at eight substrate concentrations and values of K_{cat} and K_m were determined using Eadie-Hofstee plots.

Figure 5A demonstrates that whole RNA was isolated from both normal (N) and malignant prostate cancer (T) tissues from microdissected tissue of the same prostate (*i.e.* approximately 500 mg fresh tissue for each sample). Pre-treatment of the whole RNA with the PCAM-1 DNA ZYM-1 (ZYM, SEQ ID NO:9, 31 basepair) or a random DNA sequence (31 basepair-R) failed to significantly reduce the quantity of 28S or 18S RNA visible on 1% agarose gels. The data disclosed herein (see Figure 5B) demonstrate that following RT-PCR and PCR amplification of PCAM-1 mRNA (approximately 895 bp) from the whole RNA, an approximately 895 bp transcript was amplified (after 35 cycles) from prostate cancer (T) RNA preparations but not from normal (N) or BPH RNA.

In addition, following pre-treatment of the whole RNA with the PCAM-1 DNA ZYM-1 (at 1 or 2 µg/ml for 2 hours) the mRNA was either poorly amplified (Figure 5B, lane 13) or non-existent (Figure 5B, lane 15). A random DNA oligo (31 basepair) did not prevent amplification of the PCAM-1 mRNA in control experiments, however (Figure 5B, lanes 14, 15). The data indicate that the PCAM-1 DNA ZYM-1 specifically cleaves the 5' end of the PCAM-1 mRNA to prevent primer specific amplification. Other PCAM-1 DNA ZYM-1 sequences (SEQ ID NO:10 and SEQ ID NO:11) also cleaved the PCAM-1 mRNA in a manner similar to SEQ ID NO:9)

Amino hammerhead PCAM-1 DNA ZYM 1 targeted against PCAM-1 RNA cleaved its target RNAs in a sequence-specific manner and the cleavage rates appeared to follow saturation kinetics with respect to concentration of substrate. Cleavage rates were first order at low substrate concentrations, however, as the concentration of substrate increased, the reactions rates leveled off suggesting

that PCAM-1 DNA ZYM-1 were effectively saturated with substrate. These results indicate that the cleavage reactions were truly catalytic and were therefore amenable to analysis using Michaelis Menten rate equation. From a Eadie-Hofstee plot the kinetic parameters K_m and K_{cat} were determined; PCAM-1 DNA ZYM 1 exhibited a K_m value of 87 nM and a K_{cat} value of 1.2 min^{-1}.

Under single turnover conditions, PCAM-1 DNA ZYM-1 exhibited rapid cleavage of its target sequence, the half life of the substrate being only 7 minutes. The high activity of this PCAM-1 DNA ZYM 1 is in agreement with the findings of Beigelman et al. (1995, J. Biol. Chem. 270:25701-25708), who reported that a ribozyme modified in the same manner as PCAM-1 DNA ZYM 1 exhibited almost wild type activity, with the half life of the substrate being only 3 minutes. Although cleavage was slightly slower than that demonstrated by Beigelman et al., *supra*, these findings clearly demonstrate that PCAM-1 DNA ZYM 1 is able to cleave its target in a highly efficient manner.

When the experiment was repeated using a different, non complementary, substrate control sequence, no cleavage products were detected (Figure 6), demonstrating the sequence specificity of this molecule.

Stability of PCAM-1 DNA ZYM 1 in Fetal Calf Serum

To assess the stability of the chemically modified PCAM-1 DNA ZYM 1, a comparative stability study was carried out in 100% fetal calf serum (Sigma, St. Louis, MO) at 37°C. Degradation profiles of 5' and internally [^{32}P] labeled PCAM-1 DNA ZYM 1 were compared to those of 5'-end [^{32}P] labeled phosphodiester (PO), phosphorothioate (PS) oligo-deoxynucleotides and unmodified RNA.

Synthesis/labeling

41 basepair PO and PS oligonucleotides were synthesized using an automated DNA synthesizer (model 392, Fisher, Pittsburgh, PA) using standard phosphoramide chemistry. The chemically modified 41 basepair PCAM-1 DNA ZYM 1 (Amino Hammerhead DNA enzyme) and the 15 basepair unmodified all RNA substrate were synthesized as described above. PCAM-1 DNA ZYM 1 and oligonucleotides were radiolabeled with [^{32}P] ATP and purified on 20% polyacrylamide gel as previously described.

Degradation study conditions

Radiolabeled PCAM-1 DNA ZYM 1/oligonucleotides were incubated in 100 µl of FCS at 37°C to give a final concentration of 200 nM. Ten µl aliquots were removed at timed intervals, mixed with a loading buffer containing 80% formamide, 10 mM EDTA (pH 8.0), 0.25% xylene cyanol, 0.25% bromophenol blue, and frozen at –20°C prior to gel loading. Degradation profiles were analyzed by 20% polyacrylamide (7M urea) gel electrophoresis and autoradiography. A comparative stability study was undertaken in 100% fetal calf serum (FCS) to compare the degradation profiles of 5' end labeled and internally labeled amino PCAM-1 DNA ZYM 1 to those of 5' end labeled unmodified RNA substrate, phosphodiester (PO) and phosphorothioate (PS) oligo-deoxynucleotides. The chemical modifications of the amino PCAM-1 DNA ZYM 1 resulted in a substantial increase in nuclease resistance over that of the unmodified substrate. The half life (t_{50}) of the internally labeled PCAM-1 DNA ZYM 1 was approximately 200 hours whereas the substrate was completely degraded within the time that it took to add the RNA to serum, mix and quench the reaction (t_{50} less than about 1 minute).

It was interesting to note that although the patterns of degradation were clearly different for the internally labeled PCAM-1 DNA ZYM 1 and the 5'end labeled PCAM-1 DNA ZYM 1, the kinetics of degradation were strikingly similar (t_{50} of approximately 200 hours for both).

A comparison of PCAM-1 DNA ZYM 1 degradation and oligodeoxynucleotide degradation was also performed. The chemically modified PCAM-1 DNA ZYM 1 appeared to be more stable in FCS than either the PO oligonucleotide or the PS oligonucleotide; the approximate half lives being 100 and 50 hours respectively. It must be noted, however, that the apparent degradation products migrated to the position of free phosphate. Without wishing to be bound by any particular theory, this suggests that dephosphorylation (removal of [^{32}P] label) occurred, resulting in a progressive increase in free phosphate concentration with time.

There is no doubt, however, that the findings of this study demonstrate that the chemical modifications applied to PCAM-1 DNA ZYM 1 result in an extremely stable structure. Under the conditions of this experiment

amino PCAM-1 DNA ZYM 1 proved to be the most stable to nuclease mediated degradation in fetal calf serum.

PCAM-1 DNA ZYM 1 Uptake Studies

Cell Culture Techniques

The PC-3 cell line was purchased from the ATTC collection (Bethesda, MD). These human prostate tumor cells were originally derived from bone metastasis explant technique and PC-3 ML clones were subcloned by Wang and Stearns, 1991, Differentiation 48:115-125) based on their ability to metastasize to the bone. PC-3 ML cells express the PCAM-1 at levels 10 to 50 fold higher than seen in other cell lines (Hu et al., manuscript in preparation).

The cell lines were maintained in Dulbecco's modified Eagle's media (DMEM) supplemented by 10% v/v fetal bovine serum (FBS), 1% penicillin/streptomycin and 1% v/v L-glutamine (Sigma, St. Louis, MO). The same media, without the addition of the fetal bovine serum, was used in the stability and uptake studies. CaCo-2 cells were obtained from the ATTC collection and were cultured and plated in DMEM, 10% FBS, 1% non-essential amino acids, 1% penicillin/streptomycin, and 1% L-glutamine.

Cells were cultured in 753 plastic tissue culture flasks (Fisher, Pittsburgh, PA) with 25 ml of the respective media. The cultures were incubated at 37°C in a humidified (95%) atmosphere of 5% CO_2 in air. Stock cultures were maintained by changing the media every 48 hours and the cells were passaged (1:5) when confluent (after approximately 4 days). Passaging was carried out using the following procedure: The media was removed and the cells washed with 10 ml of phosphate-buffered saline solution (PBS). Following washing with PBS, 5 ml of 2 x Trypsin/EDTA (0.25% w/v trypsin, 0.2% disodium ethylene diamine tetra-acetate in PBS, pH 7.2) was added and the flasks incubated at 37°C for 5 minutes. The flasks were tapped to dislodge the cell monolayer from the bottom and fresh media was added to neutralize the trypsin. The cells were split as required and media was added to the cells to a final volume of 25 ml.

For long term storage, frozen stock cultures were prepared in the following manner. Stock cultures were trypsinized as described above and the cells were neutralized with the addition of 10 ml of DMEM media. The cell suspension

was then transferred to a 15 ml universal tube (Fisher, Pittsburgh, PA) and centrifuged for 3 minutes at 350 revolutions per minute. The supernatant was decanted and the cell pellet was resuspended in 1 ml of freezing media (10% DMSO, 90% heat inactivated fetal calf serum) and transferred to a 2 ml screw capped cryovial (Fisher, Pittsburgh, PA). The ampoule was then placed in the freezing head of a liquid nitrogen freezer for 4-6 hours before being transferred into a liquid nitrogen (-196 °C) cell bank. When required, the cells were recovered by rapid thawing at 37°C and gradual dilution with DMEM media before seeding in 25 cm^3 flasks (Fisher, Pittsburgh, PA). The viable cell density of stock cultures was measured by haemo-cytometry using a trypan blue exclusion test. One-hundred µl of trypan blue (4 mg ml^{-1}) was mixed with 400 µl of cell suspension (1:1.25 dilution). A small amount of the trypan blue-cell suspension was transferred to the counting chamber of a Neubauer haemocytometer, with depth of 0.1 mm and area 1/440 mm^2 (Fisher, Pittsburgh, PA). The cells were counted in the 5 large squares of the haemocytometer using a light microscope. Since live cells do not take up the trypan blue dye, while dead cells do, the number of viable (unstained) cells was determined. The cell density was calculated using the following equation: cells per milliliter equals the average count per square times 104 times 1.25 (dilution factor of trypan blue).

Cell Association Studies

A series of experiments were conducted to examine the mechanism of uptake of the PCAM-1 DNA ZYM 1 in the PC-3 ML cell line. The following general experimental procedure was used throughout these studies unless otherwise stated.

Synthesis/labeling

Prior to use in uptake studies, the 41 basepair PCAM-1 DNA ZYM 1 was internally labeled with ^{32}P as previously described and purified by 20% native polyacrylamide gel electrophoresis [^{14}C] Mannitol specific activity 56 mCi/mmol) was purchased from DuPont (DuPont Chemical, Wilmington, DE)).

Uptake study procedure

PC-3 ML cells were cultured on plastic 24-well plates (Fisher,

Pittsburgh, PA). Confluent stock cultures were trypsinized and the cell density of the stock suspension diluted to 0.5 times 105 cells per milliliter with DMEM media. Each well was seeded with 2 ml of the diluted cell suspension to give a final concentration of 1 times 105 per well. The plates were incubated at 37°C in a humidified (95%) atmosphere of 5% CO_2 in air. After approximately 20-24 hours, the cell monolayers had reached confluency and were then ready for uptake experiments. The media was then removed and the monolayer carefully washed twice with PBS (2 times 1 ml times 5 minutes) to remove any traces of serum. The washing solution was aspirated and replaced with 200 µl of serum free DMEM media containing the radiolabeled DNA enzyme. Both PBS and serum free media were equilibrated at 37°C for 1 hour prior to use. The plates were incubated at 37°C, unless otherwise stated, in a dry environment for the duration of the experiment.

Once incubated for the desired period of time, the apical media was carefully collected and the radioactive content was assessed by liquid scintillation counting (LSC). The cells were then washed 3 times (3 times using 0.5 ml for 5 minutes each time) with ice cold PBS/sodium azide (0.05% w/v NaN_3 /PBS) to inhibit any further cellular metabolism and to remove any PCAM-1 DNA ZYM 1 associated with the cell surface. The washings were collected and their radioactive content was determined by LSC.

Cell monolayers were solubilized by shaking in 0.5 ml of 3% v/v Triton X-100 (Fisher, Pittsburgh, PA) in distilled water for 1 hour at room temperature. The wells were washed twice more (2 times using 0.5 ml each time) with Triton X-100 to ensure that all the cells had been harvested and that the radioactivity content of the cellular fraction was determined by LSC. Unless otherwise indicated, all experiments were performed at a final concentration of 0.01 µM ^{32}P internally labeled PCAM-1 DNA ZYM 1 and incubated for a period of 60 minutes.

The uptake of amino PCAM-1 DNA ZYM 1 was compared in different cell lines. The results demonstrate that cellular association of these PCAM-1 DNA ZYM 1 ranged from 0.42 ±0.021 ng/105 cells in NPTX-1532 normal human prostate cell lines to 1.09±0.207 ng/105 cells in PC-3 ML human prostate epithelial cells. The percent cell death was about 99% in PC-3 ML cells

and less than about 5% in NPTX-1532 cells by 48-72 hours.

The ability of PCAM-1 DNA ZYM 1 to penetrate the cell membrane and the mechanism of entrance are important considerations in developing PCAM-1 DNA ZYM 1 as therapeutics. The mechanisms by which oligo-deoxynucleotides enter cells has been well documented (for review see Akhtar & Juliano, 1991, Life Sciences 49:1793-1801) and include the involvement of fluid phase, adsorptive and receptor mediated endocytosis. The mechanism and extent of uptake is dependent on many factors including oligonucleotide type and length and cell line studied. In contrast, however, no mechanism of cellular uptake has yet been described for DNA enzymes.

PCAM-1 DNA ZYM 1 stability in PC-3 ML Cells

In order to ensure that the results obtained from the uptake studies represented cell association of intact 41 basepair PCAM-1 DNA ZYM 1 and not degraded PCAM-1 DNA ZYM 1 or free [^{32}P] label, the stability of this DNA enzyme, when incubated with PC-3ML cells, was examined. PC-3 ML cells were seeded onto 24 well-plates as previously described and used approximately 24 hours post seeding. Internally [^{32}P]-labeled PCAM-1 DNA ZYM 1 was added to 200 µl of serum free media to give a final concentration of 10 nM. Ten µl aliquots of the apical solution were collected at variable time points over a period of 4 hours, mixed with an equal volume of formamide loading buffer (9:1 v/v formamide: 1x TBE) and stored at –20°C. Prior to gel loading, the samples were heated to 100°C for 5 minutes and separated on 7M urea/20% acrylamide gels; bands were detected by autoradiography of wet gels. For comparative purposes, the stability profiles of 5' labeled PCAM-1 DNA ZYM 1, 5' end-labeled all RNA 15 basepair substrate, and 5' end labeled 37 basepair PO and PS oligo-deoxynucleotides were also measured under the same conditions.

To ensure that any findings obtained from uptake studies represented the cellular association of intact 41 basepair PCAM-1 DNA ZYM 1 and not that of shorter degraded fragments of free [^{32}P] label, the degradation of 5'-end and internally [^{32}P] labeled PCAM-1 DNA ZYM 1 was examined when exposed to PC-3 ML cells. For comparative purposes, the stability profile of an unmodified RNA substrate was also measured under the same conditions. The chemically modified

PCAM-1 DNA ZYM 1 remained largely intact throughout a four hour incubation period. While no degradation was evident from the internally labeled sample, the 5'-end labeled PCAM-1 DNA ZYM 1 did exhibit some degradation after 5 hours. Without wishing to be bound by any particular theory, this indicates that 5' dephosphorylation occurred in the latter case. In contrast, however, the unmodified RNA substrate was completely degraded within 10 minutes incubation with the PC-3 ML cell monolayer. The PCAM-1 DNA ZYM 1 was clearly protected from cellular nucleases by the chemical modifications previously described.

Optimizing PCAM-1 DNA ZYM 1 Activity

The proliferation and survival of PC-3 ML can also be inhibited by the direct addition of chemically stabilized DNA enzymes. Presumably, uptake is mediated by passive diffusion of the anionic nucleic acid across the cell membrane. In this case, efficacy could be greatly enhanced by directly coupling a ligand to the DNA enzyme. The DNA enzymes are then delivered to the cells by receptor-mediated uptake. Using such conjugated adducts, cellular uptake can be increased by several orders of magnitude without having to alter the phosphodiester linkages necessary for PCAM-1 DNA ZYM 1 cleavage activity.

Alternatively, DNA enzymes can be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, antennapae peptide coupled DNA ZYM delivery, encapsulation in liposomes, by ionophoresis, or by incorporation into other vehicles, as well as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. The DNA/vehicle combination is locally delivered by direct injection or by use of a needle, catheter, infusion pump or stent. Alternative routes of delivery include, but are not limited to, intramuscular injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of DNA enzyme delivery and administration are provided in Sullivan, et al., WO 93/23057, and Draper et al., WO 95/04818, which have been incorporated by reference herein.

Chemical modification

PCAM-1 DNA ZYM 1 sequences and PCAM-1 DNA ZYM 1 motifs

described in this invention are meant to be non-limiting examples, and those skilled in the art will recognize that other modifications (base, sugar and phosphate modifications) to enhance nuclease stability of a PCAM-1 DNA ZYM 1 can be readily generated using standard techniques and are hence within the scope of this invention.

Use of DNA enzymes Targeting PCAM-1

Over expression of the PCAM-1 has been reported in prostate cancer. Inhibition of PCAM-1 expression (for example using DNA enzymes) can reduce cell proliferation of a number of prostate tumor cell lines, in vitro and in vivo and can reduce their proliferative potential while inducing cell death (*i.e.*, greater than about 99% PC-3 ML cell death by 48-72 hours).

DNA enzymes, with their catalytic activity and increased site specificity, are likely to represent a potent and safe therapeutic molecule for the treatment of cancer. In the present invention, PCAM-1 DNA ZYM 1 does not inhibit smooth muscle, fibroblast, or normal prostate epithelial cell survival or proliferation. However, PCAM-1 DNA ZYM 1 does block and/or eradicate prostate cell tumor growth *in vivo* in SCID mice (n=31/40 mice tumors treated over 2-3 months)for a 79% response rate. In addition, mouse survival rates increased from 0% to greater than about 80% over 3-6 months. From those practiced in the art, it is clear from the examples described, that the same PCAM-1 DNA ZYM 1 can be delivered in a similar fashion to cancer cells in patients to block their proliferation and survival. Thus, PCAM-1 DNA ZYM 1 can also be used in conjunction with existing cancer therapies and physical treatments (*e.g.*, cryoablation and radiation treatment) to improve overall patient survival rates.

The data disclosed herein further demonstrate that administering ribozymes that specifically cleave RNA encoding other proteins, *e.g.*, VEGF-1 and MMP-2, further increased the therapeutic effect of administering PCAM-1 DNA ZYM 1. Thus, the present invention encompasses methods where PCAM-1 DNA ZYMs are co-administered with other ribozymes that cleave RNAs encoding proteins such as growth factors and the like.

Diagnostic Uses

DNA enzymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of PCAM-1 RNA in a cell, tissues or body fluids. The close relationship between DNA enzyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple DNA enzymes targeting the PCAM-1 mRNA, one may map nucleotide changes which are important to RNA structure and function in vitro, as well as in cells and tissues. Cleavage of target RNAs with DNA enzymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (*e.g.*, multiple DNA enzymes targeted to different genes, DNA enzymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of DNA enzymes and/or other chemical or biological molecules). Other *in vitro* uses of DNA enzymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with PCAM-1 related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a PCAM-1 DNA ZYM 1 using standard methodology.

In a specific example, DNA enzymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first PCAM-1 DNA ZYM 1 is used to identify wild-type RNA present in the sample and the second PCAM-1 DNA ZYM 1 is used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both DNA enzymes to demonstrate the relative DNA enzyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus, each analysis requires two DNA enzymes, two substrates and one unknown sample which are combined into six reactions. The presence of cleavage products is determined using an RNAse protection assay so that full-length and cleavage fragments of each RNA are analyzed in one lane of a

polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, PCAM-1) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels is adequate and decreases the cost of the initial diagnosis. Higher mutant form to wild-type ratios is correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Expression of PCAM-1 mRNA in various tissues

Northern blots using a cDNA probe which specifically recognizes PCAM-1 mRNA (*e.g.*, a probe derived from the anti-sense sequence of PCAM-1 mRNA in Figure 1A) demonstrated that PCAM-1 mRNA was expressed in PC-3 ML cells (Figure 6, lane 1), and in four human prostate cancer tissue extracts (Figure 6, lanes 2-5, respectively). More specifically, RNA isolated from PC-3 ML cells (lane 1) and dissected human prostate cancer glands from 4 different patients was examined using Northern blotting. Total RNA was isolated using TRIzol™ Reagent and then Poly(A+) RNA was isolated from the total RNA. The Northern blot was performed using ^{32}P-labeled cDNA specific for PCAM-1 according to standard methods such as those described in, among others, Sambrook et al. (1989, In: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York). Each lane was loaded with 2 micrograms of RNA.

The Northern blots with a cDNA probe which specifically recognizes PCAM-1 mRNA demonstrate that PCAM-1 mRNA was expressed in PC-3 ML cells (Figure 6, lane 1) and in 4 human prostate cancer tissue extracts (Figure 6, lanes 2-5, respectively.

Detection of PCAM-1 expression in PC-3 ML cells but not in normal NPTX-1532 cells

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of mRNA isolated from three human prostate tumors (Figure 7, lanes 1-3) and 2 human prostate tumor cell lines (*i.e.*, LNCaP and PC-3 ML cells) (Figure 7, lanes 4-

5), demonstrated that PCAM-1 mRNA was identical in size (about 1.08 Kb) to the recombinant mRNA (lane 7). Further, the data disclosed herein demonstrate that PCAM-1 mRNA was detected in three human prostate tumors (Figure 7, lanes 1-3) and in both human prostate tumor cell lines (LNCaP and PC-3 ML cell lines) (Figure 7, lanes 4-5), but was not detected in normal NPTX-1532 cell line cultured from normal human prostate glands (Figure 7, lane 6).

The data disclosed further demonstrate that the 1.08 Kb sequence of PCAM-1 mRNA was amplified using forward and reverse primer sequences including forward primer 5'-TACCCCTTGGCGCCACCGAAGGCGCCTCCAAAGCCG'3' (SEQ ID NO:3) and reverse primer 5'-CGGCTTTGGAGGCGCCTTCGGTGGCGCCAAGGGGTA –3' (SEQ ID NO:4). Thermal cycling was performed at 94°C for 1 minute, followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1.5 minutes. "M" indicates a 100 bp DNA ladder marker. Lane 8 is a negative control where SUPERSCRIPT II reverse transcriptase was omitted from the reverse transcription reaction mixture.

Quantitation and comparison of PCAM-1 mRNA expression levels in various tissues

RT-PCR of mRNA isolated from different human prostate tumors showing that the relative level of PCAM-1 mRNA expression appeared to be higher in the dissected tumor foci from Gleason Score 8 tumors (Figure 8, lanes 1-3) compared to the Gleason Score 6 tumors (Figure 8, lanes 4-6). The three different HGPIN foci (Figure 8, lanes 7-9) and BPH foci (Figure 8, lanes 10-12) examined failed to express detectable PCAM-1 mRNA, indicating that the nucleic acid was probably not expressed, or it was expressed in a very low copy number, in these glands.

The data disclosed further demonstrate that the 1.08 Kb sequence of PCAM-1 mRNA was amplified using forward and reverse primer sequences including forward primer 5'-TACCCCTTGGCGCCACCGAAGGCGCCTCCAAAGCCG'3' (SEQ ID NO:3) and reverse primer 5'-CGGCTTTGGAGGCGCCTTCGGTGGCGCCAAGGGGTA –3' (SEQ ID NO:4).

Thermal cycling was performed at 94°C for 1 minute, followed by 60 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1.5 minutes.

Effect of cell survival of PCAM-1 mRNA expression

Cell survival curves after 1-5 days in culture demonstrated that transient transfection overnight with the CMV-PCAM-1 ribozyme (ZYM-1) vector at increased concentrations (3-10 μg/ml) (Figure 9, series 3) blocked growth of PC-3 ML cells (Figure 9, series 4-7, respectively). Similar experiments using NPTX-1532 cells transfected with the CMV-PCAM-1 ZYM-1 vector failed to hinder cell growth or cell survival (Figure 9, series 2). The control studies where the PC-3 ML cells were transfected using a higher titer of the CMV-vector alone (10 μg/ml) indicated that the vector alone was not toxic even at high titers.

The nucleic acid sequence of PCAM-1 includes the sequence of SEQ ID NO:14. Further, the amino acid sequence of PCAM-1 includes the sequence of SEQ ID NO:15.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While the invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. An isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof.

2. An isolated nucleic acid encoding a mammalian prostate cancer marker 1, and homologs, variants, mutants and fragments thereof.

3. The isolated nucleic acid of claim 1, wherein said nucleic acid shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1).

4. The isolated nucleic acid of claim 1, said nucleic acid further comprising a nucleic acid encoding a tag polypeptide covalently linked thereto.

5. The isolated nucleic acid of claim 4, wherein said tag polypeptide is selected from the group consisting of a myc tag polypeptide, a glutathione-S-transferase tag polypeptide, a green fluorescent protein tag polypeptide, a myc-pyruvate kinase tag polypeptide, a His6 tag polypeptide, an influenza virus hemagglutinin tag polypeptide, a flag tag polypeptide, and a maltose binding protein tag polypeptide.

6. The isolated nucleic acid of claim 1, said nucleic acid further comprising a nucleic acid encoding a promoter/regulatory sequence operably linked thereto.

7. A vector comprising a isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof.

8. The vector of claim 7, said vector further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

9. The vector of claim 8, wherein said isolated nucleic acid encoding a mammalian prostate cancer marker 1 is expressed when introduced into a cell.

10. A recombinant cell comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof.

11. A recombinant cell comprising the vector of claim 7.

12. A recombinant cell comprising the vector of claim 8.

13. An isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation.

14. The isolated nucleic acid of claim 13, wherein said isolated nucleic acid shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1).

15. The isolated nucleic acid of claim 13, said isolated nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

16. The isolated nucleic acid of claim 15, wherein said isolated nucleic acid is expressed when introduced into a cell.

17. A vector comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation, wherein said isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, shares greater than about 98% identity with

a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1).

18. A vector comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation, said isolated nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto, further wherein said isolated nucleic acid is expressed when introduced into a cell.

19. A recombinant cell comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation.

20. A recombinant cell comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation, wherein said isolated nucleic acid shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1).

21. A recombinant cell comprising the vector of claim 17.

22. A recombinant cell comprising the vector of claim 18.

23. An isolated nucleic acid encoding a mammalian prostate cancer marker 1, wherein the amino acid sequence of said prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2.

24. The nucleic acid of claim 23, said nucleic acid further comprising a nucleic acid encoding a tag polypeptide covalently linked thereto.

25. The nucleic acid of claim 24, wherein said tag polypeptide is selected from the group consisting of a myc tag polypeptide, a glutathione-S-transferase tag polypeptide, a green fluorescent protein tag polypeptide, a myc-pyruvate kinase tag polypeptide, a His6 tag polypeptide, an influenza virus hemagglutinin tag polypeptide, a flag tag polypeptide, and a maltose binding protein tag polypeptide.

26. The nucleic acid of claim 23, said nucleic acid further comprising a nucleic acid encoding a promoter/regulatory sequence operably linked thereto.

27. A vector comprising the nucleic acid of claim 23.

28. The vector of claim 27, said vector further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

29. The vector of claim 28, wherein said isolated nucleic acid encoding a mammalian prostate cancer marker 1 is expressed when introduced into a cell.

30. A recombinant cell comprising the isolated nucleic acid of claim 23.

31. A recombinant cell comprising the isolated nucleic acid of claim 24.

32. A recombinant cell comprising the vector of claim 27.

33. A recombinant cell comprising the vector of claim 28.

34. The recombinant cell of claim 33, wherein said vector is expressed when introduced into said cell.

35. An isolated nucleic acid complementary to the nucleic acid of claim 23, said complementary nucleic acid being in an antisense orientation.

36. The isolated nucleic acid of claim 35, said complementary nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

37. A vector comprising the isolated nucleic acid of claim 35.

38. A vector comprising the isolated nucleic acid of claim 36, wherein said isolated nucleic acid is expressed when introduced into a cell.

39. The isolated nucleic acid of claim 35, wherein said nucleic acid shares greater than about 98% identity with a nucleic acid complementary with a nucleic acid having the sequence of a human prostate cancer marker 1 (SEQ ID NO:1).

40. The isolated nucleic acid of claim 39, said isolated nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

41. A vector comprising the isolated nucleic acid of claim 39.

42. A vector comprising the isolated nucleic acid of claim 40.

43. The vector of claim 42, wherein said isolated nucleic acid is expressed when introduced into a cell.

44. A recombinant cell comprising the isolated nucleic acid of claim 39.

45. A recombinant cell comprising the isolated nucleic acid of claim 40.

46. The recombinant cell of claim 45, wherein said isolated nucleic acid is expressed in said cell.

47. An isolated polypeptide comprising a mammalian prostate cancer marker 1.

48. The isolated polypeptide of claim 47, wherein said mammalian prostate cancer marker 1 shares at least about 97% sequence identity with an amino acid of SEQ ID NO:2.

49. An isolated nucleic acid that specifically binds with a prostate cancer marker 1 polypeptide.

50. The isolated nucleic acid of claim 49, wherein said nucleic acid is a double-stranded DNA.

51. The isolated nucleic acid of claim 50, wherein said isolated nucleic acid comprises a nucleic acid sequence selected from the group consisting of a nucleic acid sequence CACGGATG (SEQ ID NO:5), a nucleic acid sequence CACAATGA (SEQ ID NO:6), a nucleic acid sequence CACAATG (SEQ ID NO:7), and a nucleic acid sequence CACAATGTTTTTGT (SEQ ID NO:8).

52. An isolated nucleic acid that specifically binds with a mammalian leukemia cell break point cluster region binding protein.

53. The nucleic acid of claim 52, wherein said leukemia break point cluster region binding protein is selected from the group consisting of a Rag 1 protein and a Rag 2 protein.

54. The isolated nucleic acid of claim 53, wherein said isolated nucleic acid comprises a double-stranded DNA, said DNA comprising a nucleic acid sequence selected from the group consisting of a nucleic acid sequence CACGGATG (SEQ ID NO:5), and a nucleic acid sequence CACAATGA (SEQ ID NO:6).

55. An isolated nucleic acid that specifically binds with a prokaryotic break point cluster region binding protein.

56. The nucleic acid of claim 55, wherein said prokaryotic break point cluster region binding protein is selected from the group consisting of a RecA protein and a RecB protein.

57. The polypeptide of claim 47, wherein said polypeptide specifically binds with at least one of a nucleic acid selected from the group consisting of a nucleic acid consisting of the sequence CACGGATG (SEQ ID NO:5), a nucleic acid consisting of the sequence CACAATGA (SEQ ID NO:6), a nucleic acid consisting of the sequence CACAATG (SEQ ID NO:7), and a nucleic acid consisting of the sequence CACAATGTTTTTGT (SEQ ID NO:8).

58. An isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1.

59. The isolated enzymatic nucleic acid of claim 58, said nucleic acid comprising a nucleic acid sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11).

60. An isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein

said nucleic acid encoding a prostate cancer marker 1 comprises a nucleic acid having the sequence SEQ ID NO:1, or a portion thereof.

61. A recombinant cell comprising the enzymatic nucleic acid of claim 60.

62. The enzymatic nucleic acid of claim 58, wherein said enzymatic nucleic acid comprises binding arms and further wherein said binding arms comprise a sequence complementary to SEQ ID NO:1, or a portion thereof.

63. The enzymatic nucleic acid of claim 58, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

64. A vector comprising the enzymatic nucleic acid of claim 58.

65. A vector comprising the enzymatic nucleic acid of claim 63.

66. The vector of claim 64, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

67. The vector of claim 65, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

68. A recombinant cell comprising the vector of claim 64.

69. A recombinant cell comprising the vector of claim 65.

70. The recombinant cell of claim 61, wherein said enzymatic nucleic acid is expressed therein.

71. The enzymatic nucleic acid of claim 60, wherein said enzymatic nucleic acid is in a hairpin motif.

72. The enzymatic nucleic acid of claim 60, wherein said enzymatic nucleic acid is in a hammerhead motif.

73. The enzymatic nucleic acid of claim 60, wherein said enzymatic nucleic acid comprises a stem II region of length greater than or equal to 2 base pairs.

74. An isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein said nucleic acid encoding a prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid encoding a human prostate cancer marker 1 (SEQ ID NO:1).

75. A recombinant cell comprising the isolated nucleic acid of claim 74.

76. The recombinant cell of claim 75, wherein said isolated nucleic acid is expressed therein.

77. The enzymatic nucleic acid of claim 74, said enzymatic nucleic acid comprising binding arms wherein said binding arms comprise a sequence complementary to SEQ ID NO:1, or a portion thereof.

78. The enzymatic nucleic acid of claim 74, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

79. A vector comprising the enzymatic nucleic acid of claim 74.

80. A vector comprising the enzymatic nucleic acid of claim 78.

81. The vector of claim 79, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

82. The vector of claim 80, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

~~83. A recombinant cell comprising the vector of claim 79.~~

84. A recombinant cell comprising the vector of claim 80.

85. The recombinant cell of claim 84, wherein said enzymatic nucleic acid is expressed therein.

86. The enzymatic nucleic acid of claim 74, wherein said enzymatic nucleic acid is in a hairpin motif.

87. The enzymatic nucleic acid of claim 74, wherein said enzymatic nucleic acid is in a hammerhead motif.

88. The enzymatic nucleic acid of claim 74, wherein said enzymatic nucleic acid comprises a stem II region of length greater than or equal to 2 base pairs.

89. An isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, wherein the amino acid sequence of the prostate cancer marker 1 encoded by said nucleic acid encoding a prostate cancer marker 1 shares greater than about 97% sequence identity with the amino acid sequence SEQ ID NO:2.

90. A recombinant cell comprising the isolated enzymatic nucleic acid of claim 89.

91. The cell of claim 90, wherein said enzymatic nucleic acid is expressed therein.

92. The enzymatic nucleic acid of claim 89, wherein said enzymatic nucleic acid is in a hairpin motif.

93. The enzymatic nucleic acid of claim 89, wherein said enzymatic nucleic acid is in a hammerhead motif.

94. The enzymatic nucleic acid of claim 89, wherein said enzymatic nucleic acid comprises a stem II region of length greater than or equal to 2 base pairs.

95. An isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1 consisting of a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11).

96. A recombinant cell comprising the isolated nucleic acid of claim 95.

97. The cell of claim 96, wherein said isolated nucleic acid is expressed therein.

98. The enzymatic nucleic acid of claim 95, said enzymatic nucleic acid comprising binding arms wherein said binding arms comprise a sequence complementary to SEQ ID NO:1, or a portion thereof.

99. The enzymatic nucleic acid of claim 95, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

100. A vector comprising the enzymatic nucleic acid of claim 95.

~~101. A vector comprising the enzymatic nucleic acid of claim 99.~~

102. The vector of claim 100, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

103. The vector of claim 100, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

104. A recombinant cell comprising the vector of claim 100.

105. A recombinant cell comprising the vector of claim 101.

106. The enzymatic nucleic acid of claim 89, said enzymatic nucleic acid comprising binding arms wherein said binding arms comprise a sequence complementary to SEQ ID NO:1, or a portion thereof.

107. The enzymatic nucleic acid of claim 89, said nucleic acid further comprising a nucleic acid specifying a promoter/regulatory sequence operably linked thereto.

108. A vector comprising the enzymatic nucleic acid of claim 89.

109. A vector comprising the enzymatic nucleic acid of claim 107.

110. The vector of claim 108, wherein said enzymatic nucleic acid is expressed when introduced into a cell.

111. The vector of claim 109, wherein said enzymatic nucleic acid is expressed when introduced into a cell..

112. A recombinant cell comprising the vector of claim 108.

113. A recombinant cell comprising the vector of claim 109.

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114. An antibody that specifically binds with a mammalian prostate cancer marker 1 polypeptide, or a fragment thereof.

115. The antibody of claim 114 wherein said antibody is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a humanized antibody, a chimeric antibody, and a synthetic antibody.

116. A composition comprising an antibody that specifically binds with a mammalian prostate cancer marker 1 polypeptide, or a fragment thereof, and a pharmaceutically-acceptable carrier.

117. A composition comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, and a pharmaceutically-acceptable carrier.

118. A composition comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation, and a pharmaceutically-acceptable carrier.

119. A composition comprising an isolated polypeptide comprising a mammalian prostate cancer marker 1, and a pharmaceutically-acceptable carrier.

120. A composition comprising an isolated nucleic acid that specifically binds with a prostate cancer marker 1 polypeptide and a pharmaceutically-acceptable carrier.

121. A composition comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, and a pharmaceutically-acceptable carrier.

122. A composition comprising an antibody that specifically binds with a mammalian prostate cancer marker 1 polypeptide, or a fragment thereof, and a pharmaceutically-acceptable carrier.

123. A transgenic non-human mammal comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof.

124. A transgenic non-human mammal comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1.

125. A transgenic non-human mammal comprising an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, said complementary nucleic acid being in an antisense orientation.

126. A method of treating a disease mediated by mal-expression of a prostate cancer marker 1 in a mammal, said method comprising administering to a human afflicted with a disease mediated by mal-expression of a prostate cancer marker 1 expression-inhibiting amount of at least one substance selected from the group consisting of an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1, or a fragment thereof, an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, and an antibody that specifically binds with a mammalian prostate cancer marker 1.

127. The method of claim 126, wherein said disease is prostate cancer.

128. The method of claim 127, wherein said mammal is selected from the group consisting of a human and a dog.

129. The method of claim 127, further comprising administering an enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a polypeptide selected from a group consisting of a vascular epithelial growth factor 1 (VEGF-1) and a metalloproteinase 2 (MMP-2).

130. A method of diagnosing prostate cancer in a mammal, said method comprising obtaining a biological sample from said mammal, assessing the level of PCAM-1 in said biological sample, and comparing the level of PCAM-1 in said biological sample with the level of PCAM-1 in a biological sample obtained from a like mammal not afflicted with prostate cancer, wherein a higher level of PCAM-1 in said biological sample from said mammal compared with the level of PCAM-1 in said biological sample from said like mammal is an indication that said mammal is afflicted with prostate cancer, thereby diagnosing prostate cancer in said mammal.

131. The method of claim 130, wherein said mammal is selected from the group consisting of a human and a dog.

132. The method of claim 130, wherein said biological sample is selected from the group consisting of a prostate tissue sample, a blood sample, a urine sample, a sputum sample, a peritoneal cavity fluid sample, a perineal cavity fluid sample, a pleural cavity fluid sample, a semen sample, a prostatic fluid sample, a stool sample, and a bone marrow sample.

133. A method of diagnosing prostate cancer in a mammal, said method comprising obtaining a biological sample from said mammal, assessing the level of antibody that specifically binds with prostate cancer marker 1 in said biological sample, and comparing the level of antibody that specifically binds with prostate cancer marker 1 in said biological sample with the level of antibody that

specifically binds with prostate cancer marker 1 in a biological sample obtained from a like mammal not afflicted with prostate cancer, wherein a higher level of antibody that specifically binds with prostate cancer marker 1 in said biological sample from said mammal compared with the level of antibody that specifically binds with prostate cancer marker 1 in said biological sample from said like mammal is an indication that said mammal is afflicted with prostate cancer, thereby diagnosing prostate cancer in a mammal.

134. The method of claim 133, wherein said mammal is selected from the group consisting of a human and a dog.

135. The method of claim 133, wherein said biological sample is selected from the group consisting of a prostate tissue sample, a blood sample, a urine sample, a sputum sample, a peritoneal cavity fluid sample, a perineal cavity fluid sample, a pleural cavity fluid sample, a semen sample, a prostatic fluid sample, a stool sample, and a bone marrow sample.

136. A method of identifying a test compound that affects expression of prostate cancer marker 1 in a cell, said method comprising contacting a cell with a test compound and comparing the level of prostate cancer marker 1 expression in said cell with the level of prostate cancer marker 1 expression in an otherwise identical cell not contacted with said test compound, wherein a higher or lower level of prostate cancer marker 1 expression in said cell contacted with said test compound compared with the level of prostate cancer marker 1 expression in said otherwise identical cell not contacted with said test compound is an indication that said test compound affects expression of prostate cancer marker 1 in a cell.

137. A compound identified by the method of claim 136.

138. A method of identifying a compound that reduces expression of prostate cancer marker 1 in a cell, said method comprising contacting a cell with a test compound and comparing the level of prostate cancer marker 1 expression in said cell with the level of prostate cancer marker 1 expression in an otherwise

identical cell not contacted with said test compound, wherein a lower level of prostate cancer marker 1 expression in said cell contacted with said test compound compared with the level of prostate cancer marker 1 expression in said otherwise identical cell not contacted with said test compound is an indication that said test compound reduces expression of prostate cancer marker 1 in a cell.

139. A compound identified by the method of claim 138.

140. A method of identifying a compound that increases expression of prostate cancer marker 1 in a cell, said method comprising contacting a cell with a test compound and comparing the level of prostate cancer marker 1 expression in said cell with the level of prostate cancer marker 1 expression in an otherwise identical cell not contacted with said test compound, wherein a higher level of prostate cancer marker 1 expression in said cell contacted with said test compound compared with the level of prostate cancer marker 1 expression in said otherwise identical cell not contacted with said test compound is an indication that said test compound increases expression of prostate cancer marker 1 in a cell.

141. A compound identified by the method of claim 140.

142. A method of identifying a compound that affects binding of a prostate cancer marker 1 with a double-stranded nucleic acid that specifically binds with prostate cancer marker 1, said method comprising comparing the level of prostate cancer marker 1 binding with a double-stranded nucleic acid that specifically binds with a prostate cancer marker 1 in the presence of a compound with the level of prostate cancer marker 1 binding with said double-stranded nucleic acid that specifically binds with a prostate cancer marker 1 in the absence of said compound, wherein a higher or lower level of prostate cancer marker 1 binding with said double-stranded nucleic acid that specifically binds with a prostate cancer marker 1 in the presence of said compound compared with the level of prostate cancer marker 1 binding with said double-stranded nucleic acid that specifically binds with a prostate cancer marker 1 in the absence of said compound is an indication that said compound affects binding of a prostate cancer marker 1 with a

double-stranded nucleic acid that specifically binds with prostate cancer marker 1, thereby identifying a compound that affects binding of a prostate cancer marker 1 with a double-stranded nucleic acid that specifically binds with prostate cancer marker 1.

143. The method of claim 142, wherein said double-stranded nucleic acid that specifically binds with prostate cancer marker 1 has a sequence selected from the group consisting of a sequence CACGGATG (SEQ ID NO:5), a sequence CACAATGA (SEQ ID NO:6), a sequence CACAATG (SEQ ID NO:7), and a sequence CACAATGTTTTTGT (SEQ ID NO:8).

144. The method of claim 142, wherein said prostate cancer marker 1 has a sequence that shares greater than about 97% amino acid homology with a sequence SEQ ID NO:2.

145. A compound identified by the method of claim 144.

146. A method of monitoring the treatment of a human having prostate cancer, said method comprising:

(a) assessing the level of prostate cancer marker 1 in a first biological sample obtained from said human to determine an initial level of prostate cancer marker 1;

(b) administering an anti-prostate cancer therapy to said human;

(c) assessing the level of prostate cancer marker 1 in a second otherwise identical biological sample obtained from said human during or after said therapy;

(d) comparing said level of prostate cancer marker 1 in said first biological sample with said level of prostate cancer marker 1 in said second biological sample; and

(e) correlating any reduction in level of prostate cancer marker 1 with the effectiveness of said anti-prostate cancer therapy,

thereby monitoring the treatment of a human having prostate cancer.

147. The method of claim 146, said method further comprising repeating (b) through (e) during the course of said human's illness, anti-prostate cancer therapy, or any period or portion thereof.

148. The method of claim 146, wherein said level of prostate cancer marker 1 is assessed using a method selected from the group consisting of a method of detecting a nucleic acid encoding a prostate cancer marker 1, and a method of detecting a prostate cancer marker 1.

149. The method of claim 146, wherein said method of detecting a prostate cancer marker 1 is selected from the group consisting of a method of detecting an antibody that specifically binds with a prostate cancer marker 1, and a method of detecting binding of a double-stranded nucleic acid that specifically binds with a prostate cancer maker 1 wherein said nucleic acid is selected from the group consisting of a nucleic acid having the sequence SEQ ID NO:5, a nucleic acid having the sequence SEQ ID NO:6, a nucleic acid having the sequence SEQ ID NO:7, and a nucleic acid having the sequence SEQ ID NO:8.

150. A kit for alleviating a disease mediated by mal-expression of prostate cancer marker 1 in a mammal, said kit comprising a prostate cancer marker 1 expression-inhibiting amount of at least one molecule selected from the group consisting of an antibody that specifically binds with prostate cancer marker 1, an isolated nucleic acid complementary to a nucleic acid encoding a prostate cancer marker 1, said complementary nucleic acid being in an antisense orientation, and an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof.

151. The kit of claim 150, wherein said disease is prostate cancer.

152. The kit of claim 150, wherein said isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a

prostate cancer marker 1 comprises a sequence selected from the group consisting of a sequence GATCTTCAGGCTAGCTACAACGAGTCCTTGA (SEQ ID NO:9), a sequence AAACTTTCGACGATCGCGTCTCATCAGAAGTCCCTA (SEQ ID NO:10), and a sequence GATCTAGGGACTTCTGATGAGACGCGATCGTCGAAA (SEQ ID NO:11).

153. The kit of claim 150, further comprising an enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a polypeptide selected from a group consisting of a vascular epithelial growth factor 1 (VEGF-1) and a metalloproteinase 2 (MMP-2).

154. A kit for treating a disease mediated by mal-expression of prostate cancer marker 1 in a mammal, said kit comprising a prostate cancer marker 1 expression-inhibiting amount of at least one molecule selected from the group consisting of an antibody that specifically binds with prostate cancer marker 1, an isolated nucleic acid complementary to a nucleic acid encoding a prostate cancer marker 1, said complementary nucleic acid being in an antisense orientation, and an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof.

155. A kit for assessing the level of prostate cancer marker 1 in a sample, said kit comprising a molecule that specifically binds with prostate cancer marker 1 polypeptide or with nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof.

156. The kit of claim 155, wherein said molecule that specifically binds with a prostate cancer marker 1 polypeptide is selected from the group consisting of an antibody that specifically binds with prostate cancer marker 1, and a double-stranded nucleic acid that specifically binds with prostate cancer marker 1.

157. The kit of claim 155, wherein said nucleic acid encoding prostate cancer marker 1 shares greater than about 98% sequence identity with a nucleic acid having the sequence SEQ ID NO:1.

158. The kit of claim 157, wherein said prostate cancer marker 1 polypeptide shares greater than about 97% sequence identity with an amino acid sequence SEQ ID NO:2.

159. The kit of claim 156, wherein said double-stranded nucleic acid that specifically binds with prostate cancer marker 1 comprises a sequence selected from the group consisting of a sequence CACGGATG (SEQ ID NO:5), a sequence CACAATGA (SEQ ID NO:6), a sequence CACAATG (SEQ ID NO:7), and a sequence CACAATGTTTTTGT (SEQ ID NO:8).

160. A kit for detecting prostate cancer marker 1 in a mammal, said kit comprising a molecule that specifically binds with prostate cancer marker 1 polypeptide or with a nucleic acid encoding a prostate cancer marker 1, said kit further comprising an applicator, and an instructional material for the use thereof.

161. The kit of claims 160, wherein said mammal is selected from the group consisting of a dog and a human.

162. The kit of claim 160, wherein said molecule that specifically binds with a prostate cancer marker 1 polypeptide is selected from the group consisting of an antibody that specifically binds with a prostate cancer marker 1, and a double-stranded nucleic acid that specifically binds with prostate cancer marker 1.

163. The kit of claim 162, wherein said double-stranded nucleic acid that specifically binds with prostate cancer marker 1 comprises a sequence selected from the group consisting of a sequence CACGGATG (SEQ ID NO:5), a sequence CACAATGA (SEQ ID NO:6), a sequence CACAATG (SEQ ID NO:7), and a sequence CACAATGTTTTTGT (SEQ ID NO:8).

164. The kit of claim 160, wherein said molecule that specifically binds with a nucleic acid encoding a prostate cancer marker 1 is selected from the group consisting of a nucleic acid complementary with a nucleic acid sharing greater than 98% sequence identity with sequence SEQ ID NO:1.

~~165. A Monte Carlo-like screening assay for identification of a~~ double-stranded oligonucleotide that specifically binds with a DNA-binding protein, said assay comprising

(a) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide comprises a random core nucleotide sequence flanked by a known sequence comprising at least two basepairs; and

(b) detecting any oligonucleotide member of said set that specifically binds with a DNA-binding protein, thereby identifying a double-stranded oligonucleotide that specifically binds with a DNA-binding protein.

166. The assay of claim 165, wherein said detecting of (b) comprises a method selected from the group consisting of an electrophoretic mobility shift assay and a method of detecting a double-stranded oligonucleotide bound with a polypeptide.

167. The assay of claim 165, wherein said random core nucleotide sequence comprises from about 3 to 12 basepairs.

168. The assay of claim 165, wherein said double-stranded oligonucleotide ranges in length from about 7 to 16 basepairs.

169. The assay of claim 167, wherein said random core nucleotide sequence comprises a length selected from the group consisting of 7 basepairs, 8 basepairs, and 9 basepairs.

170. The assay of claim 165, said assay further comprising

(c) identifying the sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein;

(d) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide consists of the known flanking sequence identified in (c), said oligonucleotide further comprising an additional known such that the unknown random core sequence consists of one less unknown basepair than the sequence identified in (c), and repeating (b) and (c).

171. The assay of claim 170, said assay further comprising repeating steps (c), (b) and (c) until the entire sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein is identified.

172. An isolated double-stranded oligonucleotide that specifically binds with a DNA-binding protein identified by the assay of claim 165.

173. A method of identifying a double stranded-oligonucleotide that specifically binds with a DNA-binding protein associated with a tumor, said method comprising

(a) producing a semi-random double-stranded oligonucleotide set wherein each double-stranded oligonucleotide comprises a random core nucleotide sequence flanked by a known sequence comprising at least two basepairs;

(b) mixing a double-stranded oligonucleotide member of said set with a sample containing a mixture comprising DNA-binding proteins prepared from a tumor cell or tissue under conditions in which one or more of said double-stranded oligonucleotides in said set specifically binds a DNA-binding protein;

(c) mixing an identical double-stranded oligonucleotide member of said set with an otherwise identical sample containing a mixture comprising DNA-binding proteins prepared from an otherwise identical cell or tissue not comprising a tumor under conditions in which one or more of said double-stranded oligonucleotides in said set specifically binds with a DNA-binding protein;

(d) detecting any specific oligonucleotide-protein binding in (a) and (b); and

(e) identifying any double-stranded oligonucleotide that specifically binds with a DNA-binding protein in (b) but which does not specifically bind with a DNA-binding protein in (c), thereby identifying a double-stranded oligonucleotide that specifically binds with a DNA-binding protein associated with a tumor.

174. An isolated double-stranded oligonucleotide identified by the method of claim 173.

175. The method of claim 173, wherein said detecting of (d) comprises a method selected from the group consisting of an electrophoretic mobility shift assay and a method of detecting a labeled double-stranded oligonucleotide bound with a polypeptide.

176. The method of claim 173, wherein said random core nucleotide sequence comprises from about 3 to 12 basepairs.

177. The method of claim 173, wherein said double-stranded oligonucleotide ranges in length from about 7 to 16 basepairs.

178. The method of claim 176, wherein said random core nucleotide sequence comprises a length selected from the group consisting of 7 basepairs, 8 basepairs, and 9 basepairs.

179. The method of claim 173, said method further comprising

(f) identifying the sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein in (e);

(g) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide consists of the known flanking sequence identified in (f), said oligonucleotide further comprising an additional known basepair adjacent to said unknown random core sequence such that said unknown random core sequence consists of one less unknown basepair than the sequence identified in (f); and

(h) repeating (b) and (e).

180. The method of claim 179, said method further comprising repeating (b) through (h) until the entire sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein is identified.

181. A Monte Carlo-like screening assay for identification of a double-stranded DNA-binding protein , said assay comprising

(a) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide comprises a random core nucleotide sequence flanked by a known sequence comprising at least two basepairs; and

(b) detecting any DNA-binding protein that specifically binds with an oligonucleotide member of said set, thereby identifying a double-stranded DNA-binding protein.

182. The assay of claim 181, wherein said detecting of (b) comprises a method selected from the group consisting of an electrophoretic mobility shift assay and a method of detecting a double-stranded oligonucleotide bound with a polypeptide.

183. The assay of claim 181, wherein said random core nucleotide sequence comprises from about 3 to 12 basepairs.

184. The assay of claim 181, wherein said double-stranded oligonucleotide ranges in length from about 7 to 16 basepairs.

185. The assay of claim 184, wherein said random core nucleotide sequence comprises a length selected from the group consisting of 7 basepairs, 8 basepairs, and 9 basepairs.

186. The assay of claim 181, said assay further comprising

(c) identifying the sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein;

(d) producing a semi-random double stranded oligonucleotide set wherein each double-stranded oligonucleotide consists of said known flanking sequence identified in (c), said oligonucleotide further comprising an additional known such that the unknown random core sequence consists of one less unknown basepair than the sequence identified in (c), and repeating (b) and (c).

187. The assay of claim 186, said assay further comprising repeating steps (c), (b) and (c) until the entire sequence of the double-stranded oligonucleotide that binds with the greatest affinity with a DNA-binding protein is identified.

188. An isolated double-stranded DNA-binding protein identified by the assay of claim 181.

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FIG.1A

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FIG.1B

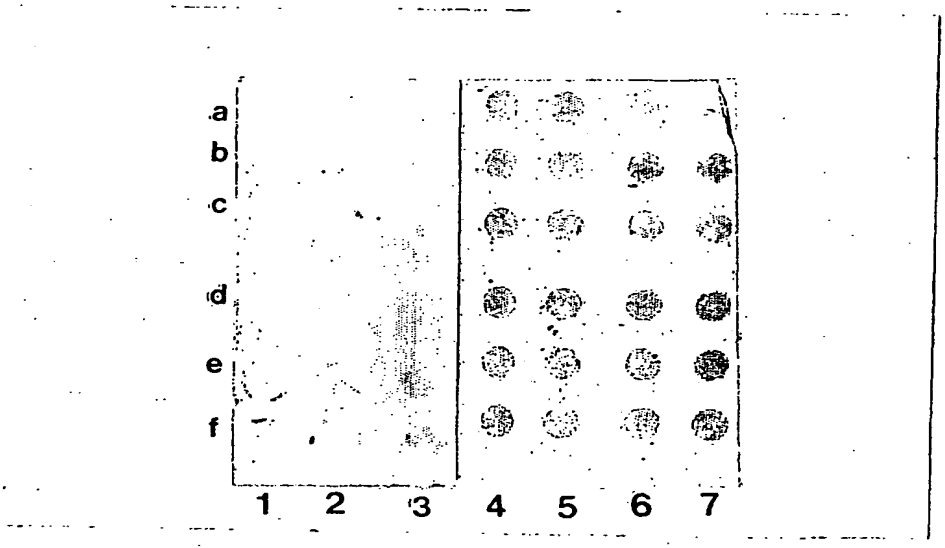
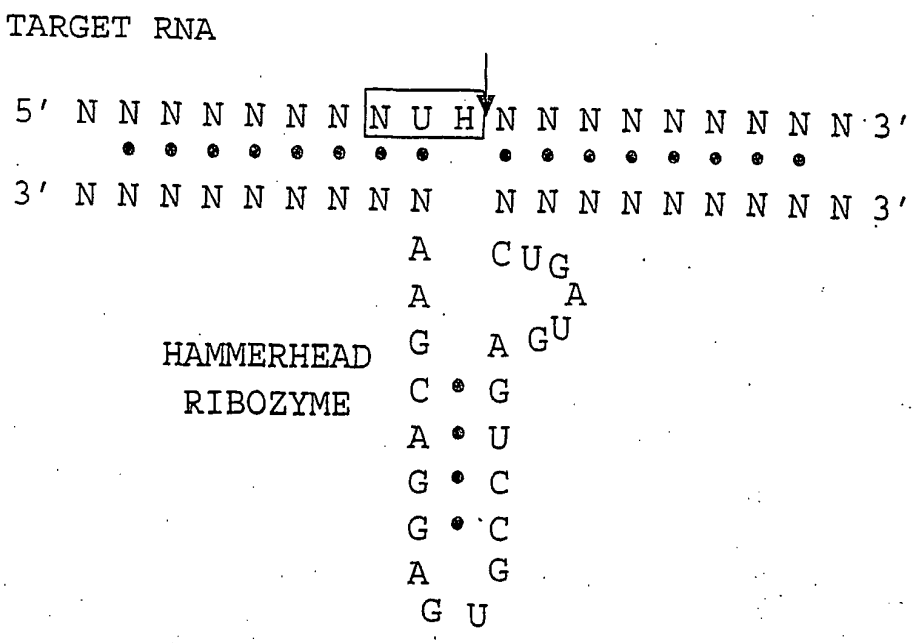


FIG.2

FIG.3



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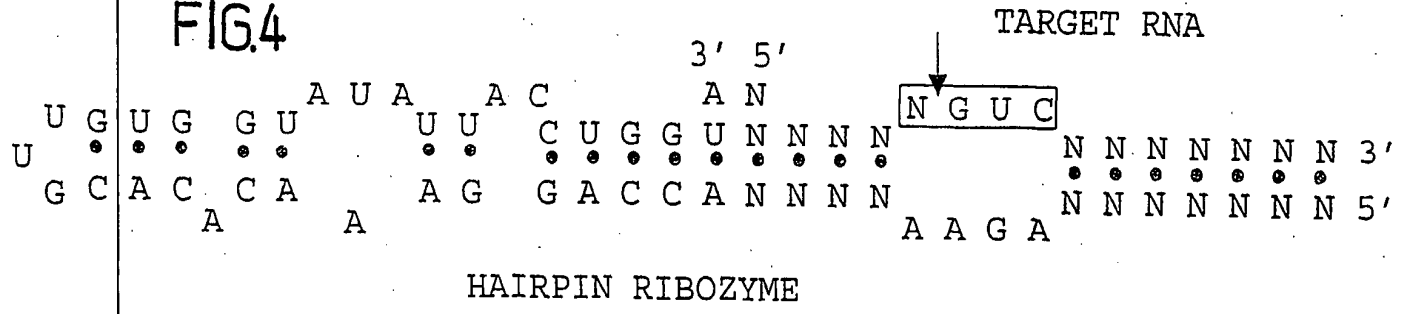

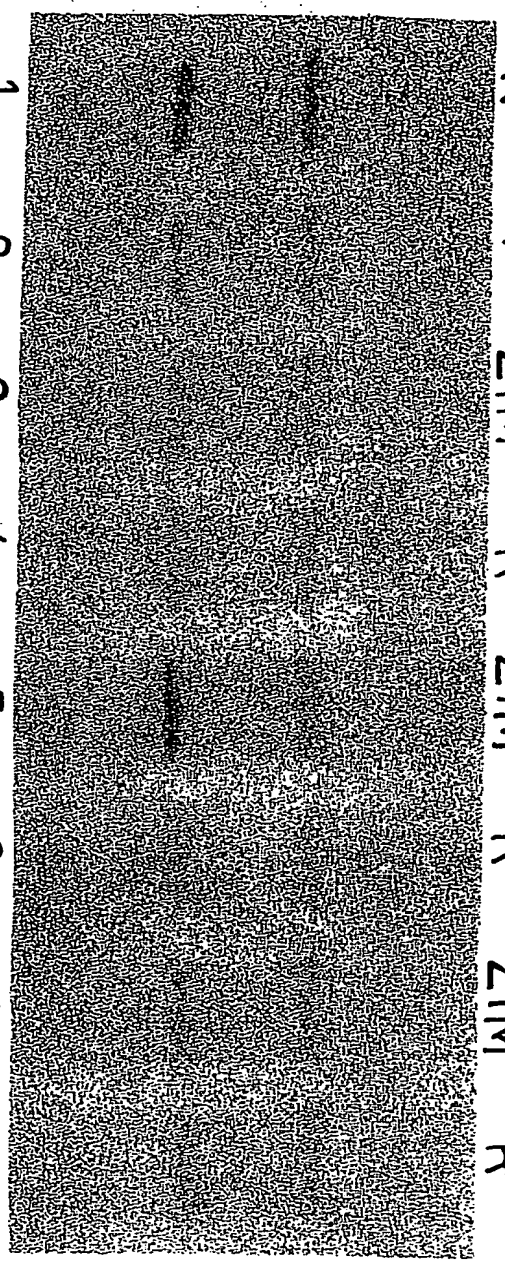

FIG.5A

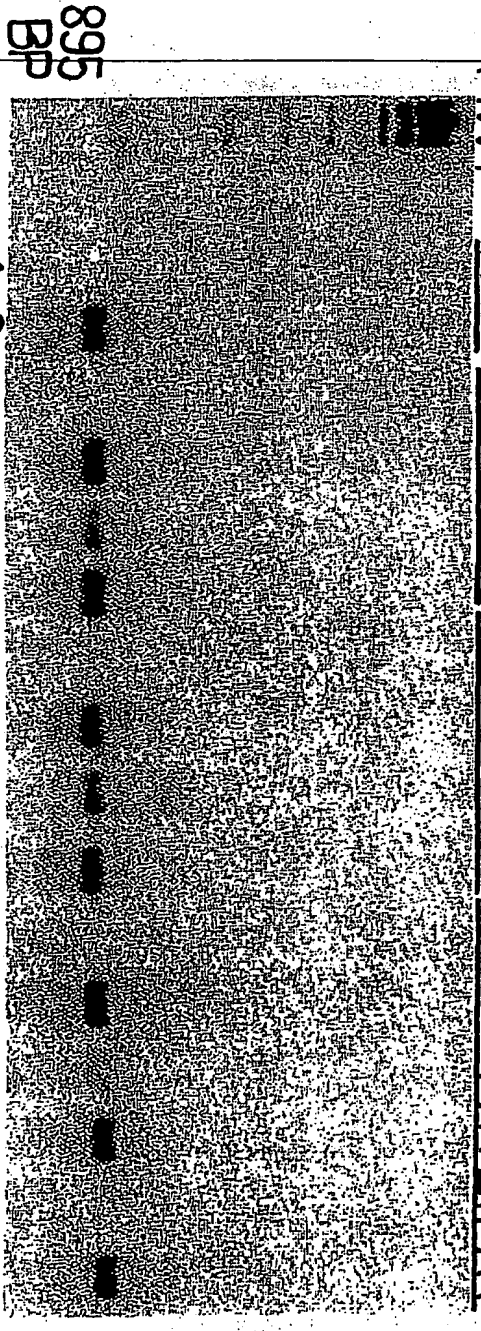

FIG.5B

RT-PCR PRODUCTS: PCAM-1 PRIMER

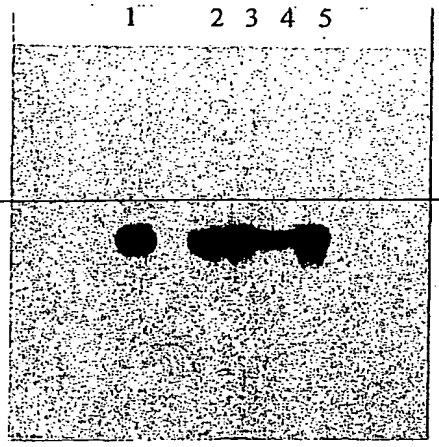

FIG.6

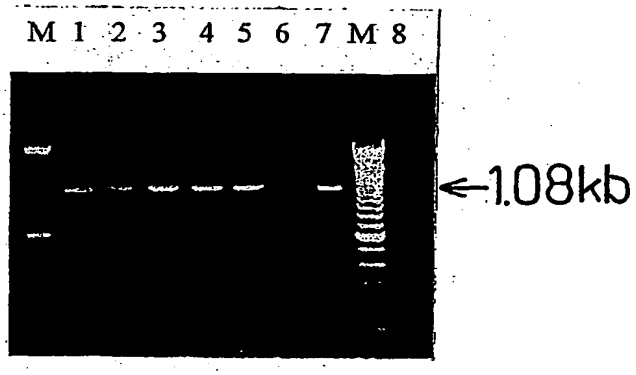

FIG.7

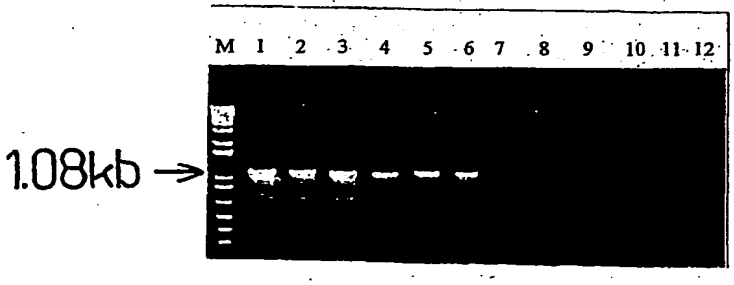

FIG.8

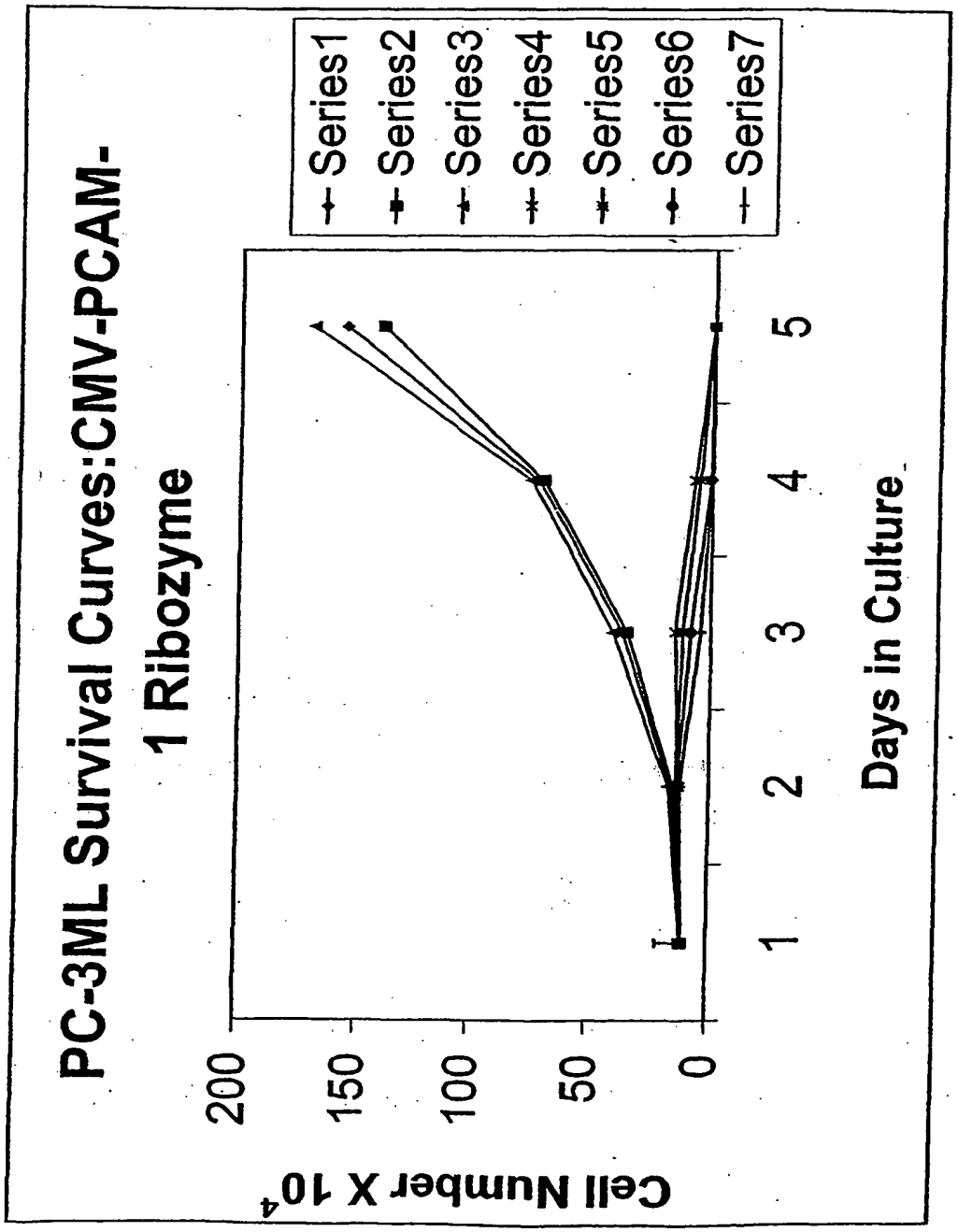

FIG. 9

SEQUENCE LISTING

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STEARNS, Mark
HU, Youji
WANG, Min

<120> PROSTATE CANCER-RELATED COMPOSITIONS, METHODS, AND KITS BASED ON DNA MACROARRAY PROTEOMICS PLATFORMS

<130> 053844-5011WO

<140> Not Yet Assigned
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<150> US 09/813,380
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(74) Agents: **ALVAREZ, Raquel, M.** et al.; Morgan, Lewis & Bockius, L.L.P., 1701 Market Street, Philadelphia, PA 19103 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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12 December 2002

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WO 02/083081 A3

(54) Title: PROSTATE CANCER-RELATED COMPOSITIONS, METHODS AND KITS BASED ON DNA MACROARRAY PROTEMICS PLATFORMS

(57) Abstract: The invention relates to novel nucleic acids encoding a mammalian PCAM-1 gene, and proteins encoded thereby, whose expression is increased in certain diseases, disorders, or conditions, including, but not limited to, prostate cancer. The invention further relates to methods of detecting and treating prostate cancer, comprising modulating or detecting PCAM-1 expression and/or production and activity of PCAM-1 poly peptide. Further, the invention relates to novel assays for the identification of DNA-binding proteins and the double-stranded oligonucleotide sequences that specifically bind with them.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/08673

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 6, 7.1, 7.21, 7.23, 69.1, 320.1; 530/300, 350, 385, 386, 387.1, 387.7; 536/1, 18.7, 22.1, 23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

GenCore sequence databases, U.S. Patent databases, WO databases

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	US 6,294,343 B1 (MACK et al) 25 September 2001, column 20, lines 10-44.	4-6
X,P --- Y,P	WO 01/21828 A1 (PHILADELPHIA HEALTH AND EDUCATION CORPORATION) 29 March 2001, entire document.	1-3, 7-12, 49, 50, 52, 53, 55, 56, 114-118, 120, 122, 130-135, 146-149, 155-157, 160-162, 164-188 --- 4-6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
09 SEPTEMBER 2002

Date of mailing of the international search report
01 OCT 2002

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
Valerie Bell-Harris for
ALANA M. HARRIS, PH.D.
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/08673

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	GenCore sequence database. 29 March 2001. Sequence comparison between Applicants' SEQ ID NO: 1 versus sequence of WO 200121828 A1.	1-4, 49, 50, 52, 53, 55, 56, 117, 118 and 120

Form PCT/ISA/210 (continuation of second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/08673

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
~~because they relate to subject matter not required to be searched by this Authority, namely:~~

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-12, 49-56, 114-117, 118 , 120, 122, 130-135 ,146-149 and 155-188

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/08673

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

A61K 35/14, 38/00; C07K 1/00, 2/00, 4/00, 5/00, 7/00, 14/00, 16/00, 17/00; C12P 21/08, 21/06; C07H 5/04, 5/06, 19/00, 21/00, 21/04; C08B 37/00; G01N 33/48; C12Q 1/00, 1/68; G01N 33/53, 33/567, 33/574; C12N 15/00, 15/09, 15/63, 15/70; 15/74

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

, 435/4, 6, 7.1, 7.21, 7.23, 69.1, 320.1; 530/300, 350, 385, 386, 387.1, 387.7; 536/1, 18.7, 22.1, 23.1, 23.5

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-12, 49-56, 117, 118 and 120, drawn to an isolated nucleic acid encoding a mammalian prostate cancer marker 1 or a fragment thereof.

Group II, claim(s) 13-46, drawn to an isolated nucleic acid in an antisense orientation complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1.

Group III, claim(s) 47, 48, 57 and 119, drawn to an isolated polypeptide.

Group IV, claim(s)58-113 and 121, drawn to an isolated enzymatic nucleic acid.

Group V, claim(s) 114-116 and 122, drawn to an antibody that binds with a mammalian prostate cancer marker 1.

Group VI, claim(s) 123-124, drawn to a transgenic non-human mammal comprising an isolated nucleic acid encoding a mammalian prostate cancer marker 1.

Group VII, claim(s) 125, drawn to transgenic non-human mammal comprising an isolated nucleic acid in an antisense orientation complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1.

Group VIII, claim(s) 126-129, drawn to a method of treating a disease mediated by mal-expression of prostate cancer marker 1 comprising administering to a human an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1. These claims will be searched with this group to the extent the method reads on the administration of a human an isolated nucleic acid complementary to an isolated nucleic acid encoding a mammalian prostate cancer marker 1.

Group IX, claim(s) 126-129, drawn to a method of treating a disease mediated by mal-expression of prostate cancer marker 1 comprising administering to a human an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1. These claims will be searched with this group to the extent the method reads on the administration of a isolated enzymatic nucleic acid.

Group X, claim(s)126-129, drawn to a method of treating a disease mediated by mal-expression of prostate cancer marker 1 comprising administering to a human an antibody that specifically binds with a prostate cancer marker 1. These claims will be searched with this group to the extent the method reads on the administration of an antibody.

Group XI, claim(s) 130-132, drawn to method of diagnosing prostate cancer in a mammal comprising obtaining a biological sample and assessing the level of PCAM-1 in said sample.

Group XII, claim(s) 133-135, drawn to method of diagnosing prostate cancer in a mammal comprising obtaining a biological sample and assessing the level of antibody that binds with prostate cancer marker I in said sample.

Group XIII, claim(s)136, 138 and 140, drawn to a method of identifying a compound that reduces expression of prostate cancer marker 1 in a cell.

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PCT/US02/08673

Group XIV, claim(s) 137, 139 and 141, drawn to a compound.

Group XV, claim(s) 142-144, drawn to a method of identifying a compound that affects binding of prostate cancer marker 1 comprising comparing the level of prostate cancer marker 1 binding with a double-stranded nucleic acid.

Group XVI, claim(s) 145, drawn to a compound.

Group XVII, claim(s) 146-149, drawn to a method of monitoring the treatment of a human having prostate cancer comprising administering an anti-prostate cancer therapy.

Group XVIII, claim(s) 150, 151 and 154, drawn to a kit for alleviating a disease comprising an antibody that binds with a prostate cancer marker 1. Said claims will be searched with this group to the extent the kit comprises only an antibody.

Group XIX, claim(s) 150, 151 and 154, drawn to a kit for alleviating a disease comprising an isolated nucleic acid complementary to a nucleic acid encoding a prostate cancer marker 1. Said claims will be searched with this group to the extent the kit comprises only a nucleic acid encoding a prostate cancer marker 1.

Group XX, claim(s) 150-154, drawn to a kit for alleviating a disease comprising an isolated enzymatic nucleic acid which specifically cleaves RNA transcribed from a nucleic acid encoding a prostate cancer marker 1. Claims 150, 151 and 154 will be searched with this group to the extent the kit comprises only an enzymatic nucleic acid.

Group XXI, claim(s) 150, 151 and 154, drawn to a kit for alleviating a disease comprising an isolated nucleic acid complementary to a nucleic acid being in an antisense orientation. Said claims will be searched with this group to the extent the kit comprises only an antisense nucleic acid.

Group XXII, claim(s)155, 156, 158 and 160-162, drawn to a kit for assessing the level of prostate cancer marker 1 comprising a molecule that binds with prostate cancer marker 1 polypeptide.
Claims 155, 156 and 160 will be searched with this group to the extent the kit comprises an antibody.

Group XXIII, claim(s) 155-157 and 159-164, drawn to a kit for assessing the level of prostate cancer marker 1 comprising a double-stranded nucleic acid that binds with prostate cancer marker 1. Claims 155, 156 and 160 will be searched with this group to the extent the kit comprises a double stranded nucleic acid molecule.

Group XXIV, claim(s) 165-171 and 181-187, drawn to a Monte Carlo-like screening assay.

Group XXV, claim(s)s 172, 174 and 188, drawn to an isolated double-stranded oligonucleotide.

Group XXVI, claim(s) 173 and 175-187, drawn to a method of identifying a double stranded-oligonucleotide comprising producing a semi-random double-stranded oligonucleotide set.

The inventions listed as Groups I-XXVI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Pursuant to 37 C.F.R. 1.475(d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention, Group I comprises the first -recited product, a isolated nucleic acid encoding a mammalian prostate cancer marker 1 or a fragment thereof.

The technical feature linking groups I-XXVI appears to be that they all relate to a prostate cancer marker 1 or fragment thereof.

However, U.S. Patent number 5,506,106 (9 April 1996) teaches an isolated nucleic acid fragment encoding a mammalian prostate cancer marker 1.

Therefore, technical feature linking groups I-XXVI does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.